REGISTERED NO. M. 3121

OF MICHIGAN
Feb. 13'61

PROCEEDINGS
OF THE
INDIAN ACADEMY
OF SCIENCES

VOL. LII] SECTION A [No. 6

THE PERCEPTION OF LIGHT AND COLOUR
AND
THE PHYSIOLOGY OF VISION

DECEMBER, 1960

PRINTED AT THE BANGALORE PRESS, [illegible]
SUPERINTENDENT, AND PUBLISHED
BY [illegible]
BANGALORE.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part I. The Mechanism of Perception

By Sir C. V. Raman

(*Memoir No.* 125 *of the Raman Research Institute, Bangalore-6*)

Received October 21, 1960

1. Introduction

Light, colour and vision play a role of fundamental importance in human life and much has been written about them from diverse points of view. Nevertheless, we do not find in the literature of the subject an acceptable answer to such a simple question as the following: How and why is it possible for us to distinguish by sight the different radiations emitted by a light source, such as the mercury arc for example, and name the colours we perceive? It is no answer to such a question to state that these radiations differ from each other in wavelength. While wave-optics enables us to understand why it is possible for well-defined images of external objects to be formed on the retinæ of our eyes, the mechanism by which the incidence of the light on the retinæ is communicated to the cerebral centres and its relation to the sensations which we perceive are subjects that stand completely outside the scope of the wave-theory of light. We have therefore of necessity to seek for an answer to our questions on the basis of the alternative approach provided by the quantum theory of radiation.

If a beam of monochromatic light falls upon a matt white surface, the illuminated object as seen by our eyes exhibits two distinct subjective features, *viz.*, its brightness or luminosity and its hue or colour. From a physical point of view, the light falling on the surface has two distinct and definable properties, firstly, the radiant energy incident on it per unit of area per unit of time, and secondly, the magnitude of the quanta or units of light energy comprised in the beam. We are therefore led to associate the two characters of subjective perception respectively with the two physical attributes of the light, in other words, luminosity with energy-flux and colour with the magnitude of the individual light-quanta, and to infer that our eyes perceive colour by reason of the fact that light energy appears in discrete

quanta and that these quanta are of different magnitude for the different rays in the spectrum. That we are justified in thus associating the sensation of colour with the magnitude of the light-quanta is reinforced by the consideration that for light to be perceived it must first be absorbed by the visual receptor and such absorption necessarily occurs in complete quanta.

We have begun by considering the case of monochromatic light for the reason that it is the simplest case: monochromatic light does not admit of any further analysis. Only then it becomes possible to indicate the correlation between the physical properties of light and our sensory perceptions of the same in an unequivocal fashion. The sensations which result from the superposition of light of different colours or of different spectral composition present problems of greater complexity. They are no doubt of scientific interest and of practical importance, but we can scarcely hope that their study would lead us to an understanding of the fundamental aspects of the perception of light and colour. On the other hand, as is well known, they have led in the past to controversies and discussions of an unprofitable nature on various questions, of which it is sufficient to mention the two following as examples: Are there only three primary colours or a larger number? Which of the colours we perceive should be regarded as fundamental and which others as derived therefrom?

2. THE DUPLICITY OF HUMAN VISION

Our eyes are capable of functioning and serving us usefully over an enormous range of intensities. It has been estimated that the illumination of horizontal ground by the night-sky without moon is only about a hundred-millionth part of that received in direct sunlight, and yet it suffices for finding our way and keeping to the path provided our eyes are well adapted to darkness. That the mechanisms which function and enable us to perceive light are identical at both ends of this vast range of illumination is scarcely to be believed. Indeed, as is well known, the character of the sensations evoked is not the same in the two cases. In night-vision our eyes perceive differences in luminosity but their appreciation of colour is obviously imperfect. There are also other features which distinguish day-vision from night-vision, but we shall not pause here to discuss them.

In the year 1866, the retinologist Max Schultze announced a conclusion to which he had been led by an extended series of investigations in comparative ocular histology. He noted that the retinæ of nocturnal vertebrates had many rods, and few cones or even none, while diurnal species had retinæ with many cones which might even lack rods entirely. Thus,

arose the idea that the cones are the receptors for daylight-vision and the rods for night-vision. That two types of vision exist and must be distinguished is very generally recognized, and they have indeed been given special names, *viz.*, photopic and scotopic vision respectively. In the literature of the subject it is also generally assumed that photopic vision is mediated by the cones and scotopic vision by the rods in the retinæ. But this assignment of the two types of vision to two sets of receptors in the retina does not really take us far. We have to ask ourselves whether the physical and physiological processes are the same in the two types of vision or whether they are different and if so, in what respects.

In night-vision, our eyes have to serve us at the very lowest levels of illumination and a supersensitive receptor is clearly necessary for their successful functioning in such circumstances. On the other hand, in daylight vision, the levels of illumination are higher; indeed, the quantity of light available in day-vision is, judging by the standards obtaining in night-vision, enormously larger than is adequate for its effective perception. Hence, a relatively insensitive receptor is what is actually needed. A further aspect of the matter that should be taken note of is that our eyes have to serve us both at very low and at very high levels of illumination, though, of course, not simultaneously. It would evidently be desirable that the supersensitive mechanism of night-vision is put out of action when the insensitive mechanism needed for daylight-vision is functioning; it would, of course, then be necessary that the apparatus of night-vision is restored and put back into action when it is actually needed. No such switching off and on is needed in the case of daylight-vision.

The foregoing remarks make it clear that the mechanisms of photopic and of scotopic vision would differ in certain respects. The exact nature of such difference is however best discussed at a later stage after the actual facts regarding the photopic and scotopic perception of light have been reviewed.

3. The Facts of Colour Perception

From our present point of view, the most important facts concerning colour and daylight-vision are the relationships which exist between the subjectively perceived sensations of luminosity and colour and their physical counterparts, *viz.*, the total energy-flux and the magnitude of the energy-quanta appearing in different parts of the visible spectrum. We may first briefly recall the distribution of colour in the spectrum. The colours observed in it differ so obviously from each other that common usage finds it convenient to give them different names to aid us in recalling them to our

minds, *viz.*, red, orange, yellow, green, blue and violet. The ranges over which these designations are applied vary with the individuals concerned, and only a kind of general average can therefore be regarded as possessing any significance. Expressed in wave-numbers and therefore directly related to the magnitude of the energy-quanta, these ranges are, respectively, Red (13,000–16,000), Orange (16,000–17,000), Yellow (17,000–17,600), Green (17,600–20,200), Blue (20,200–23,000) and Violet (23,000–26,000). It is noteworthy that while the ranges in wave-numbers covered by the designations red, green, blue and violet are all of the same order of magnitude, the colours designated as orange and yellow cover much smaller ranges.

We shall return presently to a more detailed discussion of the colour sequence observed in the spectrum, and proceed meanwhile to consider the relation between the subjectively perceived brightness in different parts of the spectrum and the physically measurable energy-distribution in it. The essential features of this relationship can be established in a very simple manner by holding a diffraction grating in front of the eye and viewing the straight filament of a tungsten lamp, the current flowing through which is controlled by a rheostat. Observations of the first-order spectrum into which the luminous filament appears drawn out show that the distribution of luminosity in it is quite different from the energy-distribution in thermal black-body radiation as given by the Planck formula. According to the latter formula, the wavelength of maximum energy in the spectrum lies in the remote infra-red when the source has a temperature of 500° K., and though it moves towards the red end of the visible spectrum as the temperature is raised, it remains outside the visible spectrum even at as high a temperature as 3,000° K. On the other hand, the maximum luminosity as visually observed appears well within the spectrum even when the temperature of the filament is so low that it glows only feebly with a red colour. As the temperature of the filament is raised and it becomes brilliantly incandescent, the point of maximum luminosity in the spectrum moves swiftly towards shorter wavelengths and finally reaches a point in the greenish-yellow region of the spectrum at about 5600 angstroms beyond which it ceases to move any further. This is also the wavelength of maximum visual intensity in the spectrum of direct sunlight. In the spectrum of the light of the blue sky, in which the energy-distribution relative to sunlight is radically altered, the position of the maximum visual intensity is nevertheless not appreciably different. It is evident from these facts that it is a characteristic of daylight-vision that the eye has a highly pronounced maximum of visual sensitivity at about 5600 angstroms on either side of which the visibility of radiation falls off rapidly. It is also evident from the observations that

such falling off of visibility is less rapid towards the red end of the spectrum than towards the violet.

The features referred to above find quantitative expression in the "relative luminous efficiency" curves for monochromatic radiations appearing in the visible spectrum which have been determined by several investigators. The two methods most often used for this purpose are the so-called step-by-step method and the flicker method. In the former method, the difficulties of heterochromatic photometry are minimised by dividing up the spectrum into a fairly large number of strips (twenty or more) and successively comparing the intensities of adjoining strips. In the other method, the luminosity of monochromatic radiation is determined by the method of comparison at the rate of 10–20 times per second against a constant source of white light, the intensity of the monochromatic light being varied until the flicker experienced is a minimum. Differences are noticed between the determinations made by different observers, but by averaging the results obtained with a large number of observers, a luminous efficiency curve is obtained which can be regarded as a property of normal human vision. The results obtained by the two different methods are about the same, though some discrepancies are noticeable. This is shown by the two curves reproduced as Fig. 1 below exhibiting the results obtained by Gibson and Tyndall with the step-by-step method, and by Coblenz and Emerson with the flicker method.

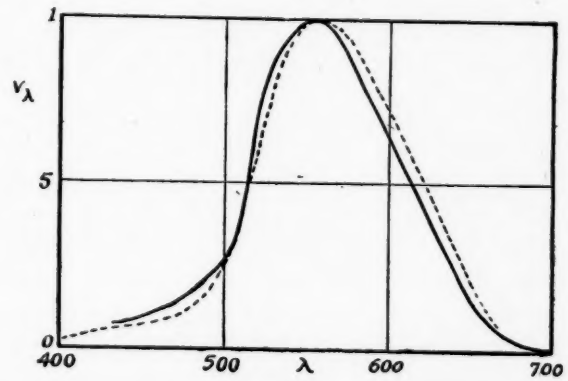


FIG. 1. Relative luminous efficiency curves by the step-by-step method (——) and the flicker method (- - - - -).

We now return to the colours exhibited by monochromatic light. Earlier, it was indicated that the varying colours of the spectrum represent

our subjective perception of the physical fact that the energy-quanta are different in its different regions. On this basis, we should expect the colours of the spectrum to form a continuous sequence, in other words that the spectrum can be divided into a great many parts or sections of which the colour can be perceived to be different. That is indeed actually the case. The number of distinct patches in the spectrum which when set side by side cannot be matched by any alteration in relative brightness has been estimated as about 250. If all the sections into which the spectrum may thus be divided were of equal width on a frequency scale, the smallest spectral shift giving rise to an observable change in colour would be about 50 wave-numbers. If this figure were accepted, it would mean that the human eye possesses a most remarkable degree of sensitivity to differences in colour and that it is capable of appreciating a difference less than one-half of one per cent. in the magnitude of the light quantum received and absorbed by the retina as a difference in colour of the radiation.

It should be remarked, however, that the minimum spectral shift needed for a noticeable change of colour is not the same at all parts of the spectrum. It exhibits very significant variations, being in some parts smaller and in other parts larger than the figure stated above. Numerous investigations have been made in the past to determine the minimum in various parts of the spectrum and when the results reported are compared with each other, one finds a general agreement regarding certain features, *viz.*, that in the region around 4900 angstroms and also in the region around 5900 angstroms, the minimum has quite small values, being respectively about 9 angstroms and 11 angstroms, and that in the intermediate region, *viz.*, around 5400 angstroms, it is distinctly larger, being about 21 angstroms. Expressed in wave-numbers, these same figures are, respectively, 38 and 26 and 62 wave-numbers as against the averaged figures of 50 stated above. At wavelengths less than 4900 and greater than 5900 angstroms the determinations show that the minimum shift of wavelength increases rapidly. In these regions, however, the quantitative results of different investigators differ rather widely from each other.

The method by which the data of the kind referred are obtainable may be briefly described as follows: The observer views the two halves of a photometric field illuminated respectively by light from two adjacent regions in the spectrum of which the separation can be varied. The relative brightness of the two halves of the field is adjusted to equality by the use of a rotating sectored disk or by some other device. If, when so matched, the two parts of the field appear definitely different, the spectral shift giving

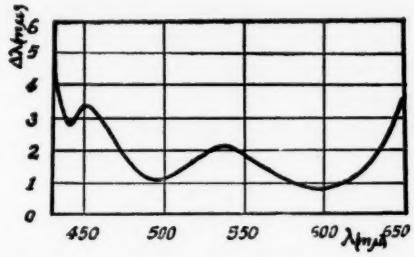


FIG. 2. Hue discrimination curve in the spectrum, according to Wright and Pitt.

rise to the difference represents the least perceptible to the eye as a difference in colour. Figure 2 above exhibits "the hue discrimination curve" in the spectrum obtained in this manner and reported by two observers. The abscissæ in the curves are the wavelengths of monochromatic light in millimicrons, while the ordinates are the smallest shifts of wavelength (also in millimicrons) giving rise to an observable difference in colour. The figure exhibits the features already referred to above, and another not there mentioned, *viz.*, the existence of a marked dip in the hue discrimination curve at about 4400 angstroms. Most of the published investigations report this feature and there is no doubt that it represents a well-established result.

Quite apart from the results of specific measurements made by the methods referred to above, it should be stated that a simple inspection of the spectrum by the unaided eye indicates that the colour sequence in the spectrum changes rapidly in certain regions, less rapidly in others and much less rapidly in certain others. An observer with normal colour vision will notice that the change of the blue to the green of the spectrum takes place within a relatively small range of wavelengths between, say, 4900 and 5000 angstroms. Likewise, he will notice that the transition from the yellow to the orange of the spectrum takes place between, say, 5900 and 6000 angstroms. On the other hand, the transition from green to yellow is continuous, so much so that it is difficult to locate with any exactness the boundary between these two colours. The boundary between the blue and violet regions of the spectrum is placed in very different positions by different observers and indeed, it would seem that Newton was not altogether without justification when he put an additional colour "indigo" between blue and violet.

4. THE MECHANISM OF PHOTOPIC VISION

We now proceed to consider the significance of the facts regarding colour perception set forth above. For the reasons already explained, we

associate the sensation of luminosity with the physical intensity or energy-flux, and the sensation of colour with the magnitude of the energy-quanta comprised in the radiation. Both the luminosity and colour of monochromatic radiation admit of study by appropriate physical methods which lead to quantitative results. These results do not contradict the physical interpretations proposed and they accordingly require to be further examined and explained. In other words, we have to answer various questions concerning them. In the first place, why does the luminous efficiency of monochromatic radiation vary in the manner observed and exhibit a maximum around 5600 angstroms? What is the factor which limits the power of the eye to discriminate differences in colour in the different parts of the spectrum? Why is it as great as it actually is in some parts, and why it is smaller in others?

The human eye has often been compared with a photographic camera. There are indeed some resemblances; in both cases we have a light-tight box covered inside with dark pigment to absorb unwanted light, a shutter to exclude light when desired and an adjustable diaphragm to regulate its admission when necessary. In both cases also, we have an optical system with front and rear components capable of adjustment so as to form a focussed image at the rear of the box. But to stress these structural similarities between a photographic camera and the human eye is liable to make us forget that the basic purposes which they are intended to serve are fundamentally dissimilar. A photographic camera serves to make a record of the light image on the sensitive film which can be developed and preserved. On the other hand, the retina of the eye serves both as a receiver and as a transmitter to the cerebrum of the energy of light falling on it. Whereas a photographic film can be used only once, the retina of the human eye is always ready for use. Indeed, vision would be useless to us if it did not carry with it the ability to view objects in different directions in quick succession one after another.

It is self-evident that light which merely passes through the retina and is absorbed by the pigmentary epithelium behind it would be ineffective in vision. In other words, the absorption of light by pigments present within the retina itself is the first and necessary step for vision to be possible. But it is equally evident that unless such absorption goes hand in hand with a retransmission of the absorbed energy to the cerebral centres through the optic nerves, vision cannot result. It should also be remarked that since the colour perceived is determined by the magnitude of the energy-quanta comprised in the radiation, we have to assume that each quantum of energy

absorbed is transferred immediately to the neurons without addition or subtraction. It would not be possible otherwise to explain the remarkable precision actually exhibited by our sense of colour discrimination. Merely as an example of such sensitivity, it may be mentioned that the two components of the sodium doublet whose wavelengths are 5890 and 5896 angstroms can be perceived to be of different colours in appropriate circumstances.

The facts of colour perception thus lead us to a quite simple and definite picture of the functioning of the retina in photopic or day-vision. We postulate that the light-sensitive receptors in the retina contain certain pigments which possess the power to transfer the light-energy absorbed by them to the neurons and return to their state of lowest energy. The known facts regarding the propagation of nervous disturbances and the production of electrical potentials by the infall of light on our eyes suggest that the energy transferred from the pigments assumes the form of an electrical disturbance in the receptors which is conducted through the optic nerves and on reaching the cerebral centres there excites the sensations we perceive. But we shall not pursue this aspect of the matter any further here.

5. The Characters of Scotopic Vision

We now proceed to consider the actual facts regarding the perception of light at low levels of illumination, also referred to as scotopic vision. Earlier in this memoir it was suggested that the duplicity of human vision calls for a mechanism which could switch off the apparatus of scotopic vision when there is ample illumination and photopic vision can therefore function, and would switch it on again when the illumination is very feeble and photopic vision ceases to be possible. That such a mechanism is actually provided is evident from our own sensations when we move from sunshine into a dark chamber and *vice-versa*. The switching-on process is the slower of the two, since it is necessary to remain in the dark for at least thirty minutes for our eyes to develop the full sensitivity characteristic of scotopic vision. The switching-off process is somewhat quicker, but its duration is found to depend on the intensity of the light to which the retina is exposed.

Earlier, it was mentioned that in scotopic vision, the colour sense is imperfect. Such imperfection is due largely to the circumstance that the great sensitivity to light characteristic of scotopic vision does not extend to the parts of the spectrum which are both luminous and colourful, *viz.*, the red and orange regions. Indeed, when any object of a scarlet-red colour

is viewed in a darkened room, it appears quite black. The characteristic features of scotopic vision are however best appreciated by studying the appearance of a continuous spectrum in scotopic conditions. A simple and convenient way of doing this is for the observer in a dark room to examine sky-light entering the room through a long vertical slit through a glass diffraction grating held in front of his eye. If the observations are made in day-time, the first-order diffraction spectrum of the slit then seen exhibits the familiar features characteristic of photopic vision. If, on the other hand, the observation is made at night when the sky is lit by moon-light (or in the absence of the moon by the lights of the city), the spectrum appears greatly shortened by the absence of all wavelengths greater than about 6000 angstroms. By making the observations during the twilight hours (evening or morning), the progressive change from the photopic to the scotopic spectrum or *vice-versa* can be readily followed. The change-over can be hastened or slowed down by the simple method of narrowing or widening the slit through which sky-light enters the dark room, or by the observer moving away from or towards the slit. In either case, large changes in the visual intensity of the spectrum result and the entire series of changes from the photopic to the scotopic conditions can be quickly and conveniently followed. Indeed, one can go down to the stage in which the intensity of the spectrum approaches the threshold of vision.

A characteristic feature of the spectrum seen under scotopic conditions is that the wavelength of maximum visual intensity shifts towards the blue as compared with photopic vision. Quantitative determinations of the relative luminous efficiency curve at low levels of illumination have been made by several observers, the method used being that of direct photometric comparison against a white field of low luminosity. A typical set of results is shown graphically in Fig. 3. It will be seen that the relative luminous efficiency becomes negligible at wavelengths greater than about 6000 angstroms, while its maximum appears at about 5100 as against 5600 angstroms in photopic vision.

A question of special interest is whether the spectrum seen under scotopic conditions exhibits the colour sequence seen in photopic vision (apart from the disappearance of the longer wavelengths) or whether the scotopic spectrum is achromatic as is often stated. Observations made by the methods described above gave a clear answer to the issue here raised. Scotopic vision covers a wide range of intensities differing at its two ends by a large factor; whether in any particular case, we are observing the spectrum under scotopic conditions is indicated by the relative luminosity of the regions

around 5000 and 6000 angstroms, the latter being negligible in scotopic conditions, while in photopic conditions, the latter is much more intense than the former. While the scotopic spectrum exhibits no visible colour anywhere at the lowest levels of intensity, this ceases to be the case at greater intensities. A greenish tinge becomes visible around the region of maximum luminosity; this is more obvious at greater intensities, and the differences in colour between the centre of the spectrum and its two ends are also then perceptible. As we approach the upper limits of scotopic vision, the colours observed become more vivid, but remain distinctly weaker and less saturated than in photopic vision.

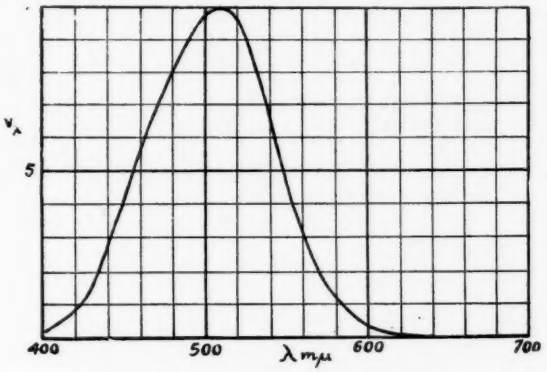


FIG. 3. Relative luminous efficiency in scotopic vision.

6. THE MECHANISM OF SCOTOPIC VISION

In their fundamental aspects, scotopic and photopic vision resemble each other closely. It is just as easy to view different objects one after another in quick succession at low levels of illumination as at higher levels and indeed one is then less troubled by after-images, glare and other disturbances. Visual photometry is possible in scotopic as in photopic vision and indeed in some respects easier, as for instance in heterochromatic photometry. If scotopic vision had been entirely achromatic at all levels of illumination, this feature would differentiate it fundamentally from photopic vision. But, as we have seen, such is not the case. The colour sense is actually present at the higher levels of illumination falling within the scotopic range and the colours then perceived, though less vivid, do not otherwise differ from those seen in photopic conditions.

In view of what has been stated, one would be justified in assuming that in their basic aspects, the mechanisms of scotopic and photopic vision

are similar, in other words that in either case, the retinal pigments function by a simple transference of the light energy absorbed by them to the receptors, followed by a return to their normal energy state. The differences between the characters of photopic and scotopic vision described earlier do not appear to be inconsistent with such an assumption. That the relative luminous efficiency curve for scotopic vision differs from that determined for photopic vision is readily understood on the basis that the retinal pigments which function in the two cases are different. That scotopic vision goes down to lower levels of illumination also becomes intelligible on the basis that the retinal pigment which functions is present in substantial quantities and can therefore trap a larger proportion of the incident energy quanta and pass it on to the receptors.

It now remains to explain how the switching-off and switching-on of scotopic vision take place respectively when the observer moves from low levels of illumination to high levels and *vice-versa*. It may justifiably be inferred from the actual facts of observation that when the observer has been long enough in bright light, the pigment which mediates scotopic vision has been transformed by the incidence of such light on his retina into a different substance which cannot so function; *vice-versa*, when the observer remains in darkness long enough, the pigment is regenerated in some fashion. The switch-off would proceed at a rate proportional to the intensity of light to which the retina is exposed and would be the quicker, the more intense such illumination is. The regeneration of the pigment, on the other hand, would occur in the absence of light or at very low levels of illumination and its rate would be determined by purely chemical or biochemical factors. The destruction of the pigment by bright light and its regeneration in the dark here contemplated are merely auxiliary processes which make it possible for human vision to function efficiently both at high and at low levels of illumination. There is no need and indeed no justification for assuming that they form an essential part of the actual mechanism of scotopic vision. Neither would photopic vision demand the destruction and regeneration of the visual pigments which function in it as an essential part of the visual mechanism.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part II. The Visual Pigments

BY SIR C. V. RAMAN

(Memoir No. 125 of the Raman Research Institute, Bangalore-6)

Received October 21, 1960

1. INTRODUCTION

IN the first part of this memoir, the view has been put forward that our perception of light and colour is made possible by the presence in the retina of certain pigments which possess the power to absorb light and to transfer the energy thus absorbed immediately to the sensory receptors, thereby enabling us to perceive the absorbed energy as light. The identification of the visual pigments which perform these functions, in other words the determination of their chemical nature and a knowledge of their spectroscopic behaviour and distribution in the retina are the foundations on which any explanation of the facts of experience regarding vision must rest. We shall address ourselves in this part of the memoir to the problem which thus confronts us.

2. VIEWING THE RETINA IN ACTION

Any observer endowed with normal colour vision can perform the experiments now to be described. They do not require elaborate equipment and enable him to see his own retina in the act of functioning and observe its response to light in different parts of spectrum. From the results of the experiments, it is possible to infer the number and the distribution in the retina as well as the spectroscopic behaviour of the visual pigments which enable us to perceive light and colour.

The technique of the observations is quite simple. The observer sits facing a brightly illuminated white screen at a convenient distance from it and views the screen through a colour filter, either monocularly or binocularly as he may find best suits him. After allowing a sufficient time for the vision to adapt itself to the effects of the colour filter, the observer fixes his vision on some particular point on the screen and suddenly removes the filter. He will then recognize on the screen a highly enlarged projection of the macular region of his own retina, including especially the fovea

centralis and the foveola at its midpoint. The details of the picture seen and the colours it exhibits depend very much on the filter employed. The picture soon fades away but it can be restored by putting back the filter and removing it again at short intervals of time. The picture can then be examined more closely.

The appearance of an image of the observer's own retina on the screen can be explained in general terms in the following manner. The colour filter employed cuts out or enfeebles certain parts of the spectrum while transmitting the other parts of it freely. As a consequence of the removal of the filter, the spectral components which are cut out or enfeebled by it are restored and suddenly illuminate the retina. Localised sensations are then excited, determined by the luminous efficiency of these spectral components and by the response to them of the receptors located in each element of area of the retina. The sensations thus excited manifest themselves to the sensory mechanism of the eye as an image of the retina which appears projected on the screen, each element of area exhibiting its own response to the illumination suddenly falling on it.

Before we proceed to describe and comment on the results observed with individual filters, it may be useful to mention a few particulars requiring attention in the experiments. It is necessary that the illumination of the screen should be adequate and that it should be as nearly uniform as possible. The observations should therefore be made by daylight, the screen being placed facing the windows in a well-lighted room and the observer should sit facing the screen and with his back to the windows. A projection screen of the well-known kind which is plastered over with tiny glass spheres is very suitable. A medium-sized screen, say 170 cm. by 125 cm., is adequate and a convenient distance from the observer to a screen of that size is 350 cm. It is possible to use colour filters of different kinds, as for example glass cells of sufficient size containing absorbing solutions or plates of coloured glass. However, for the studies now under consideration, the most convenient filters are those which can be prepared by staining gelatine films on glass (of the kind used in photography) with a water-soluble dye. By regulating the strength of the solution in which the plates are dipped and the period of immersion before they are taken out and allowed to dry, the depth of the staining can be controlled over a wide range. The filters should be held close to the eye so as to exclude all light except that passing through it.

3. DESCRIPTION OF THE EFFECTS OBSERVED

Quite spectacular effects are observed when the filter employed is a gelatine film on glass stained lightly with methyl-violet. The filter appears

a purplish-blue by transmitted light. Holding it before the eye for a few seconds and then removing it, the observer sees on the screen an enormously magnified image of his own fovea as a disk of light which is green in colour, and at the centre of it a bright spot of the same hue which is the pit or depression in the fovea known as the foveola. If the filter is held for a somewhat longer time before it is removed, the fovea is much brighter and then appears surrounded by a halo of golden-yellow hue some five or six times larger in diameter but less luminous than the fovea itself. The fovea with the foveola at its centre appears at the point on the screen at which the observer has fixed his vision before removing the filter. If he shifts his gaze, they also move, thereby showing clearly that what is seen on the screen is a projected image of the observer's own retina. (See Colour Plate, Fig. I.)

Spectroscopic examination shows that the filter lightly stained by methyl-violet has a strong absorption band in the orange, a weaker one in the green, and a very weak absorption in the region between them. When more heavily stained, the absorption becomes complete in all these regions and extends further into the green. A film stained heavily so that it cuts out the green completely but transmits the red and the blue of the spectrum appears of a deep purple colour. The use of such a heavily stained filter is however of no particular advantage, and on the other hand makes the phenomena rather less spectacular.

A noteworthy fact that emerges from the studies is that a filter whose absorption spectrum appears *exclusively* in the green part of the spectrum, in other words between 4900 and 5600 angstroms, does *not* give any observable effects of the kind referred to above. Several colour filters of this kind are available, amonsgt which may be mentioned very dilute solutions of potassium permanganate in water, or of iodine in carbon tetrachloride. More convenient, however, are gelatine films stained lightly by a suitable dye as, for example, rhodamine or eosine, so as to enfeeble or cut out the green region without affecting the rest of the spectrum. Holding such a filter in front of the eye for a little while and then removing it, the screen presents the same appearance as before the filter is interposed. Prolonging the period for which the filter is held before the eye prior to its removal makes no noticeable difference.

Though, as stated above, colour filters that absorb only the green part of the spectrum do not give rise to the effects under consideration, it is clear from the example of the methyl-violet filter that absorption in the green

region can co-operate with absorption in adjoining regions of greater wavelengths and enable them to be observed. We may therefore conveniently divide the spectrum into three regions which we may denote as A, B and C and classify the filters and the effects they produce by reference to the regions of the spectrum in which their absorption appears. The violet-blue region of the spectrum is denoted by A, the green by B, and the yellow, orange and red may be grouped together and referred to as C.

As an illustration of the usefulness of the classification proposed in relation to the effects observed, we may here mention two cases in which very beautiful effects are observed but of a different nature. The colour filters in both cases are aqueous solutions contained in glass cells of equal thickness, five centimeters in each case. One solution is that of nickel chloride and the other of the dye-stuff lissamine-green sufficiently diluted so as to transmit plenty of light. The nickel chloride solution is completely transparent in the green, and examination by a pocket spectroscope shows that it also allows the adjoining part of the blue to pass through, but cuts out the red, orange and yellow more or less completely. It thus belongs to the class of filter which we denote as AC. The solution of lissamine-green appears blue-green by transmitted light and while resembling the nickel chloride in allowing part of the blue to come through, it cuts outs the red, orange and yellow completely and also part of the green. It thus belongs to the class ABC.

Holding the nickel chloride solution before the eye for a few seconds and then removing it, a brilliant rose-red glow is seen to cover the entire screen; at the centre of the field and as a very inconspicuous feature appears a small circular area which appears of a different hue and brightness from the rest of the field. On the other hand, when the lissamine-green solution is used for the observations, a highly magnified image of the fovea, bright yellow in colour and more luminous than the rest of the field, and the foveola at its centre are the most conspicuous features which the observer sees on the screen. A reddish glow covers the entire screen, but around the foveal image appears a halo of indefinite hue. (See Colour Plate, Fig. II.)

The filters of the species designated as A and as AB transmit the red, orange and yellow regions of the spectrum perfectly but cut off the shorter wavelengths to varying extents. As their cut-off in the spectrum moves from the extreme violet into the green and then further, the colour of the transmitted light progressively alters from the palest yellow to the deepest orange. All filters of the kind mentioned exhibit effects having certain

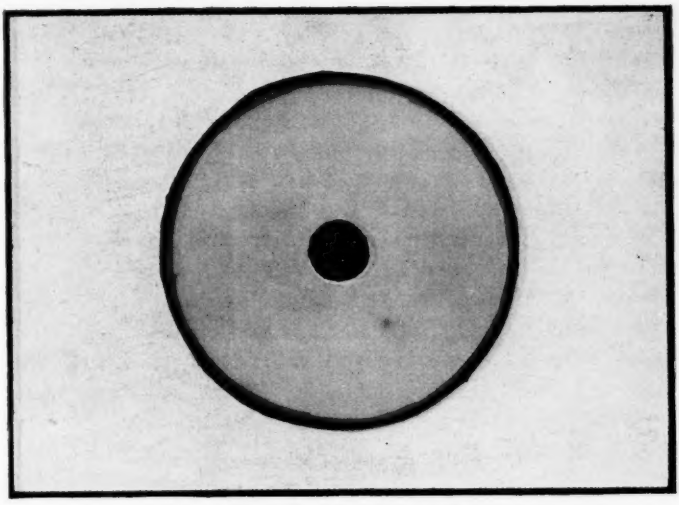


Fig. 1 Methyl Violet Filter

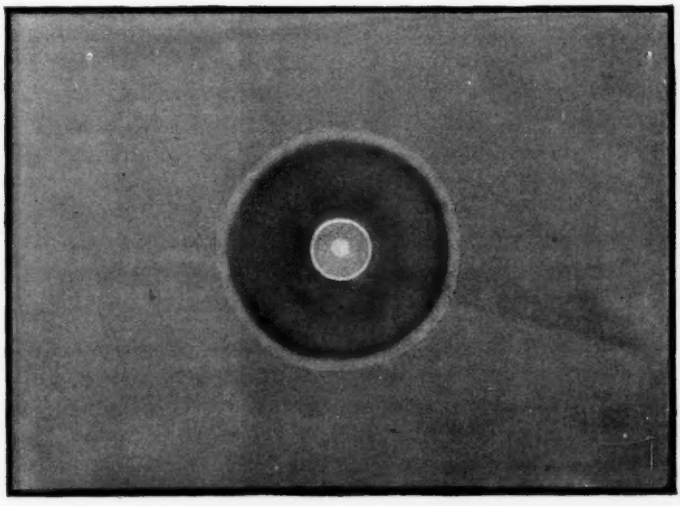


Fig. 2 Lissamine Green Filter

Pictures of Retina seen by the Filter Technique.

features in common, though differing in detail. For example, using a filter of pale yellow hue having a cut-off at 4500 angstroms, its sudden removal from before the eye after an adequate interval results in the appearance of a violet-tinted glow which covers the entire screen. Neither the fovea nor the foveola nor any halo surrounding them can be seen. But at the centre of the field in the direct line of the observer's vision, there is a hint of the glow being somewhat dimmer than elsewhere on the screen. With another filter of a deep yellow colour which has a cut-off at 5100 angstroms, similar effects are noticed, but the glow which appears covering the screen is blue in colour and much brighter than that observed with the pale yellow filter. Orange filters give effects which are similar to those seen with the deep yellow filter but are not by any means more conspicuous; on the other hand, the effects are rather less conspicuous since the glow does not display any vivid colour and also ceases more quickly to be observable. Thus, an extension of the region of absorption by the filter from section A of the spectrum into section B does not make any real difference to the results, apart from making them less easy of observation.

Gelatine filters of the classes C and AC may be prepared by staining them *very lightly* with the dyes methylene-blue and lissamine-green respectively. Holding such filters before the eye and then removing them, the entire area of the screen exhibits a glow of a colour which differs for the two species of filter, orange in one case, and rose-red in the other. But no image of the fovea or foveola appears on the screen. Only filters of the classes BC and ABC exhibit the later phenomenon. They may be prepared in great variety by staining gelatine films to any desired depth by blue or green, dyes (methylene blue and lissamine-green for example). Most blue glasses belong to the class BC and most green glasses to the class ABC. A photographic filter exhibiting a delicate green colour was however found to belong to the class AC. It cuts out the violet rays in the spectrum and visibly enfeebles the red, but has no noticeable absorption in any other part of the spectrum. Holding it before the eye and then removing it, the observer sees a beautiful purple glow covering the entire screen, while an inconspicuous feature appears at the centre of the field of vision in the shape of a small circular area where the glow is feebler than elsewhere.

Observations have been made with numerous filters of the classes BC and ABC. With every one of them, the foveal disk and the foveola at the centre are conspicuous features. It is also possible in many cases to observe a coloured halo five or six times larger in diameter than the foveal disk but

of much lower intensity superposed on the general field of illumination on the screen following the removal of the filter.

4. THE THREE VISUAL PIGMENTS

We now proceed to consider the significance of the facts of observation reported above. We have seen that the entire spectrum may be divided into three sections which we have denoted as A, B and C respectively, and that the colour filters whose absorption appears exclusively in one or another of these three behave quite differently. It is a reasonable interpretation of the facts that the retina contains three pigments which are effective as absorbers of light and mediators of vision respectively in these three sections. We shall refer to them hereafter as pigments A, B and C respectively. Pigment A functions in the blue-violet region of the spectrum, pigment B in the green and pigment C in the orange, red and yellow. The facts of observation described earlier, however, compel us to qualify this statement and to recognize that the absorption spectrum of pigment C overlaps that of pigment B in the green to an appreciable extent. It is also necessary to assume that when the incident light causes pigment C to function, this results in the simultaneous functioning of pigment B in the same region of the retina, provided the wavelength of the incident light is within the region of overlap of the absorption spectra of the two pigments.

The effects observed on the viewing screen following the removal of the filter enable us to draw some useful inferences regarding the distribution in the retina of the visual pigments which give rise to them. The position is clearest with regard to pigment A which is the mediator of vision in the blue-violet region of the spectrum. The blue or violet glow covering the entire area of the screen seen with filters of class A clearly indicates that the visual pigment of that class is distributed over an extent of the retina many times larger in area than the fovea itself.

The appearance on the viewing screen of a highly enlarged and luminous image of the fovea and of the halo encircling it clearly demands the co-operation of the two pigments B and C, since these effects are only noticed when filters of the classes BC and ABC are employed. The colours displayed by the fovea and the halo in various cases also point to the same conclusion. We are thus led to infer that pigment B is present in its maximum density in the region of the fovea and has a considerable though smaller density in an area surrounding the fovea and having five or six times its diameter. The observations further indicate that pigment C is present in association with pigment B in the areas referred to and that it is also to be

found distributed over the area of the macula well beyond the region in which the coloured haloes surrounding the fovea are observed.

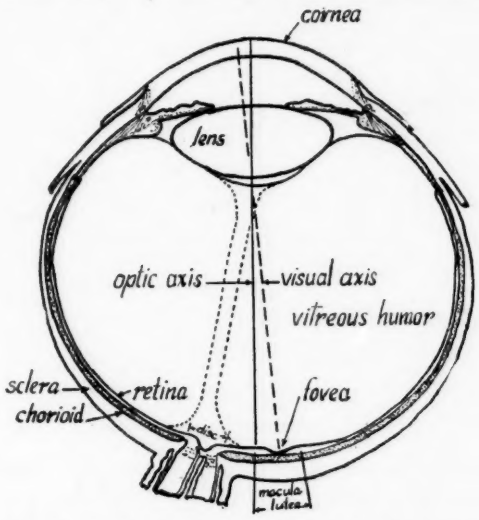


FIG. 1. Horizontal section of the right human eye.

We may reasonably look for some evidence in support of the foregoing findings in the appearance of the retina as seen in the ophthalmoscope. The fundus of the human eye is visible in that instrument by reason of the diffusion of the light incident on the retina by the retina itself and by the materials behind it. The opinion expressed in the ophthalmological treatises (and copied therefrom into other books) is that the appearance of the retina is due to the diffusion of light by the choroid coat which lies behind the retina. Between the retina and the choroid coat, however, lies the pigment epithelium, the function of which is to absorb unwanted light traversing it in either direction. The efficiency of the pigment epithelium as an absorbing screen has therefore a very great influence on the nature of the picture seen in the ophthalmoscope. This is indeed evident on a comparison between the appearance of the retina in the four cases of albinotic individuals, persons of fair complexion, and persons of dark and very dark complexions respectively. The blood-vessels in the choroid are clearly seen through the retinæ of albinotic individuals. They are not seen in the other cases, what is actually visible being the surface of the retina upon which can be distinguished the optic disk, the retinal blood-vessels and the macula. The hue exhibited by the retinal surface is orange-red in persons of fair complexion, while in the case of negroes it is brick-red.

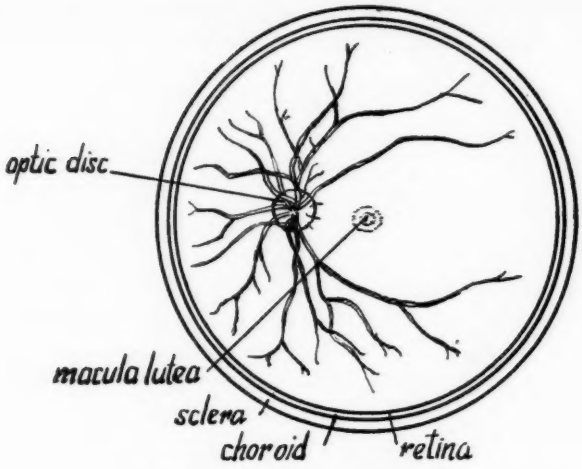


FIG. 2. Fundus of the human eye.

What we are here specially interested in is the macular region which is physiologically the most important part of the fundus. This region is devoid of any visible blood-vessels, though it is encompassed on all sides by the twigs reaching down to it from the retinal blood-supply system. A specially significant fact is that in all cases, the macular region is of a distinctly darker tint than the rest of the fundus. Further, the fovea itself exhibits a blood-red hue in all cases. These are conspicuous features in the coloured illustrations of the fundus appearing in the ophthalmological treatises. The presence of pigments which between them cover the entire spectrum would explain the deeper colouring of the macular region. The presence in and around the fovea of a pigment which exercises a powerful absorption in the green region of the spectrum would account for that area exhibiting a blood-red hue. Thus, what is actually seen of the fundus through the ophthalmoscope does not contradict the conclusions reached through the present studies, but on the other hand gives them the clearest possible support.

A further remark may be made here. Ophthalmologists not infrequently in their examination of the fundus make use of light from which the red rays have been excluded by a suitable filter. It is stated that the background of the retina then appears of a yellowish-green colour, the macula standing out as a lemon-yellow area, and that the blood-vessels running through the retina appear almost black with sharply defined outlines. That the macula is distinguishable by its colour from the rest of the

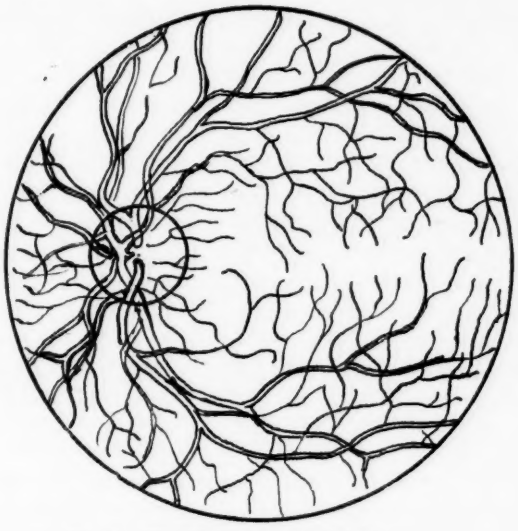


FIG. 3. Blood-vessels in the retina.

retina in these circumstances has been explained as due to presence in it of appreciable quantities of the yellow pigment which gives to the macular region its anatomical name of *macula lutea*. This yellow pigment may reasonably be identified with our pigment A, the absorption of which appears exclusively in the blue-violet region of the spectrum.

FIG. 4. Perifoveal capillaries in the retina.

5. IDENTIFICATION OF THE VISUAL PIGMENTS

We now proceed to consider the problem of determining the chemical nature of the pigments present in the retina which are the mediators of photopic vision. We have designated them above as (A), (B) and (C) and formed a general idea of the spectral ranges within which they are operative. They should be capable of existing in association with the materials which form the living substance of the retina. Their absorption spectra should appear in the regions indicated and it is necessary that when they absorb light radiations, they should also be capable of passing on the energy thus absorbed immediately to the receptors in the retina. Finally also, since we are concerned with photopic vision and therefore with high levels of illumination, it is necessary that the pigments should possess in a reasonable measure the power to resist disruption or decomposition by the action of light.

The advances in organic chemistry made of recent years have resulted in the elucidation of the structure of numerous naturally occurring colouring matters and created a great body of knowledge connecting chemical constitution with optical behaviour. A well-established result which emerges from these researches is the relation between the manifestation of colour by a substance and the presence in its molecules of unbroken chains of alternate single and double bonds. It is found that the longer the chain of such bonds is, the further into the region of the visible does the absorption spectrum of the substance extend. Many naturally occurring pigments also fall into two classes, one in which the chain of alternate single and double bonds is an extended straight line, and the other in which it forms a closed ring within the molecule. The group of substances known as the carotenoids belong to the first class, while in the second class we find various substances playing highly important biological roles, including especially heme and chlorophyll. It is interesting and significant that the green leaves of growing plants contain colouring matters belonging to both of the classes referred to. Two of them, *viz.*, β-carotene and xanthophyll belong to the first class and two others, *viz.*, chlorophyll-*a* and chlorophyll-*b* to the second.

As is well known, the pigments present in green leaves form the pathway by which organised nature finds access to solar energy. They enable the energy of solar radiation to be utilized for the synthesis from carbon dioxide and water of carbohydrates and other constituents of living matter. The mechanism of this process—known as the photosynthesis by plants—has been the topic of numerous investigations. It is recognized that chlorophyll with the strong absorption of light which it exhibits at the extreme red end

of the spectrum plays a leading role in photosynthesis and the view formerly prevailed that the other pigments present did not participate in the process. More recent studies indicate that the latter conclusion needs modification and that the carotenoid pigments also do play a part in photosynthesis. Cogent evidence is also forthcoming that the pigments in green leaves, including especially chlorophyll, act as energy-receiving and transferring agents and thus enable photosynthesis to take place. Whether they actually participate in the chemical changes which result in the formation of new compounds and if so in what manner are questions which at the present time remain in the speculative stage.

It would seem, therefore, that the pigments in green leaves and the pigments in our retinæ play somewhat similar roles in their respective fields of activity. Hence, there are good grounds for assuming that the pigments concerned are either the same or else are very similar. Four pigments are needed for vision, three for photopic and one for scotopic vision. Two of them may well be carotenoids, one for each type of vision. There is no reason, however, for assuming that all four pigments are carotenoids. On the other hand, it is most improbable that this would be the case. Pigment B plays a role in human vision of special importance as it covers the part of the spectrum having the highest luminous efficiency. One would naturally expect that such a pigment would be a product of biological activity in the human organism and not a substance carried into the body through the medium of food products. Further, it is difficult to believe that any carotenoid would possess all the properties needed for pigment B, *viz.*, an absorption spectrum lying in the region between 5000 and 6000 angstroms, a high efficiency as an energy-receiving and transferring agent and the chemical stability needed to resist disruption or decomposition under intense illumination. We are thus led to infer that pigment B belongs to the class of organic compounds which derive their colour from the presence in their molecular structure of a ring of alternating single and double bonds. That pigment C also belongs to that class is *prima facie* very probable.

Chlorophyll-*a* and chlorophyll-*b* are pyrrole pigments which are chemically very similar to each other. They owe their colour to a combination of four pyrrole nuclei joined together in a complex molecule, a special feature of importance being that a magnesium atom appears linked to the nitrogen atoms of the four pyrrole groups in the molecule. When the magnesium atom is removed by the action of acids, the solution turns brownish and ceases to exhibit the colour characteristic of chlorophyll, thus demonstrating the importance of the metallic atom in relation to its spectroscopic behaviour. When we seek for a pigment which could perform in

the retina a role analogous to that performed by chlorophyll-*a* in the green leaf, the obvious and indeed the only choice is the metallo-porphyrin compound known as ferroheme (or simply as heme). This is also a pyrrole pigment, being a compound of iron in the divalent state with one of the porphyrin group of compounds. The porphyrins contain a closed ring of eighteen bonds which are alternately single and double in their structure, and the iron atom occupies in heme a position in relation to the four nitrogens in the molecule which is the same as that of the magnesium atom in the chlorophyll structure. There is thus good reason for assuming that heme possesses the properties needed to enable it to function very efficiently as a visual pigment in the retina.

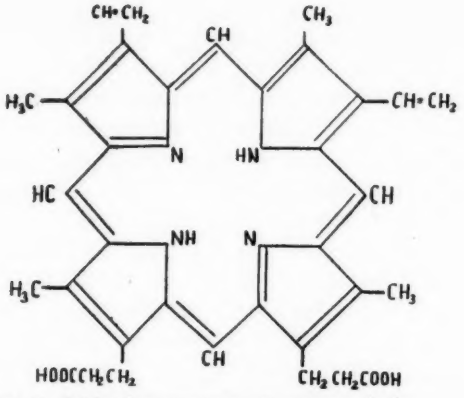


FIG. 5. Molecular structure of protoporphyrin.

It is appropriate here to mention that heme in combination with different proteins plays a role of outstanding importance in the life of both plants and animals. The compound of heme with globin known as hemoglobin is present in the red-blood cells of vertebrates and by its ability to combine reversibly with oxygen without undergoing oxidation performs the unique and indispensable role of a carrier for the storage and transport of oxygen in the organism. Another heme-protein known as myoglobin is the respiratory pigment occurring in muscle cells of both vertebrates and invertebrates. The cytochromes are the most widely distributed of all the heme proteins and occur in the cells of nearly all aerobic organisms, both plant and animal. Catalase and the peroxidases are other heme proteins which function as enzymes. This brief statement indicates the ubiquitous nature and extraordinary versatility of the heme proteins in performing biological roles of importance.

We shall later discuss the spectroscopic behaviour of heme in detail. It will suffice here to mention that all the heme proteins exhibit an intense absorption of light in the green region of the spectrum. Hemoglobin, for example, exhibits even in very dilute solutions a powerful absorption in the region of wavelengths between 5000 and 6000 angstroms, its maximum appearing around 5550 angstroms. That this absorption is a characteristic property of the porphyrin groups of compounds when a divalent metal atom occupies the central position in the molecule is shown by the fact that a powerful absorption with its maximum located at or near the same wavelength is also exhibited by several different porphyrins in combination with diverse metallic atoms which are divalent.

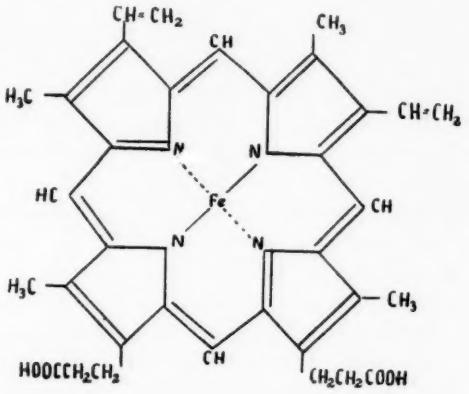


FIG. 6. The molecular structure of heme.

The considerations set forth above accordingly lead us to identify ferroheme as our pigment B. We thereby add to the list of the many biological functions performed by ferroheme that of being the principal mediator of vision in the photopic range. Indeed, the absorption of light by ferroheme is so strong that no great quantity of this pigment would be needed in the retina to enable it to function effectively as the mediator of vision. That it is indeed the principal visual pigment finds strong support in the fact that the position of its absorption maximum is not very different from the wavelength of maximum visual sensitivity in the spectrum. The generally accepted value for the latter is 5550 angstroms, but it may actually be a little greater for persons with normal colour vision.

That the pigment A which functions in the region of wavelengths between 4000 and 5000 angstroms is a carotenoid is indicated by the fact

that the absorption spectra of carotenoids appear just in that region. As already mentioned earlier, the two carotenoids that are invariably found in the green leaves of growing plants are β-carotene and xanthophyll. These colouring matters are also present in the plasma of human blood and in human milk in varying amounts, evidently as the result of the consumption of articles of food containing them. There is however a noteworthy difference between the two pigments, *viz.*, β-carotene is a precursor of vitamin-A, whereas xanthophyll is not. In other words, xanthophyll does not undergo fission and become transformed to vitamin-A in the human body. We may, therefore, with confidence assume that it is xanthophyll, and not β-carotene which is the pigment that finds its way into the human retina to function as a receptor in photopic vision.

The identification of pigment C has next to be considered. We have already noticed that while the absorption spectrum of this pigment appears principally in the yellow, orange and red regions of the spectrum, it also overlaps to some extent the region in which pigment B is operative, namely, the green of the spectrum. The reasons which make it probable that pigment C belongs to the same group of pyrrole compounds as pigment B have already been indicated. That the two pigments co-operate with other in producing the luminous effects described earlier on these pages is also significant. We are thus led to recognize that pigment C is closely related to pigment B. The suggestion that it is only an oxidised form of that pigment, in other words that it is ferriheme naturally presents itself. The spectroscopic and other evidence which supports and confirms this identification will be presented in a subsequent part of the present memoir.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part III. The Carotenoid Pigment

By Sir C. V. Raman

(*Memoir No.* 125 *from the Raman Research Institute, Bangalore-6*)

Received October 21, 1960

1. Introduction

In the first part of this memoir, the facts of observation regarding the luminosity and colour perceived at different points in the spectrum were set out in detail. The mechanism of perception and the role played by the visual pigments in the retina which would account for the facts were then discussed. In the second part of the memoir, the number of visual pigments which function and the spectral regions in which they are effective were deduced from certain luminous effects which were described, and the considerations which enable these visual pigments to be identified were also set out.

We shall now proceed a step further and show how it is possible to connect the observed variations of luminosity and colour in the spectrum with the absorption characteristics of the visual pigments which enable us to perceive light and colour. We shall in the first instance consider the blue-violet region of the spectrum which exhibits several features of interest, including especially the fact that in that region—and no other—the unaided eye is capable of recognizing polarised light and even of determining its plane of vibration by sight. It will be shown that these and other features are explicable in terms of the structure, spectroscopic behaviour and disposition in the retina of the molecules of the carotenoid pigment xanthophyll which was identified in the second part of the memoir as the mediator of photopic vision in that part of the spectrum.

2. The Carotenoid Pigments

The carotenoids are so named by reason of their chemical relationship to carotene which is a plant pigment first isolated in crystal form from the roots of the cultivated carrot. They are a numerous family of compounds and are essentially plant products. Indeed, there is no evidence

which would suggest that any carotenoid is produced *de novo* within the body of an animal. They enter the body by way of the articles of food which are consumed by the animal and if assimilated, pass into the blood stream to be utilised or stored up where needed. The potential vitamin-A activity is an important function of some of them, including especially β-carotene. This is however not one of the potential uses of xanthophyll since it is not a precursor of vitamin-A. Accordingly, xanthophyll is either accumulated in certain organs or else is passed out of the body.

An interesting example of the storage of xanthophyll is furnished by the yolk of the domestic hen's egg which, as is well known, exhibits a bright yellow colour. It is a convenient source-material from which xanthophyll can be obtained for the study of its spectroscopic behaviour. Repeated treatment with warm acetone results in the extraction of the pigment and on filtration, a clear liquid exhibiting a golden-yellow colour is obtained. This contains xanthophyll and also its isomer zeaxanthin as a minor constituent. As the latter has a very similar spectroscopic behaviour, its presence is not inconvenient for the purpose in view.

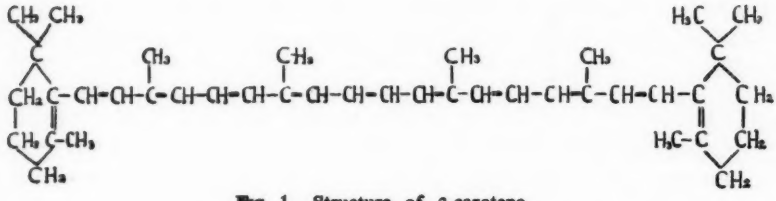


FIG. 1. Structure of β-carotene.

Carotene is a hydrocarbon having the chemical formula $C_{40}H_{56}$ which appears in different isomeric forms, the most important of them being β-carotene which has the structure shown in Fig. 1. It will be seen that the two end-groups in the molecule have an identical structure and are joined together by a long chain in which single and double bonds alternate. It is this system of conjugated double bonds that is responsible for the absorption of light by the substance which extends into the visible region of the spectrum. The characteristics of the absorption spectrum of β-carotene in hexane solution are exhibited in the spectrophotometer curve reproduced as Fig. 2. It will be seen that it is negligible at wavelengths greater than 5200 angstroms and that it rises very steeply in the region of wavelengths smaller than 5000 angstroms. After passing through a succession of maxima, it drops down but much less rapidly to quite small values at the violet end of the spectrum. The absorption maxima are located at 4770,

4500 and 4250 angstroms, the last being visible only as a point of inflexion on the curve.

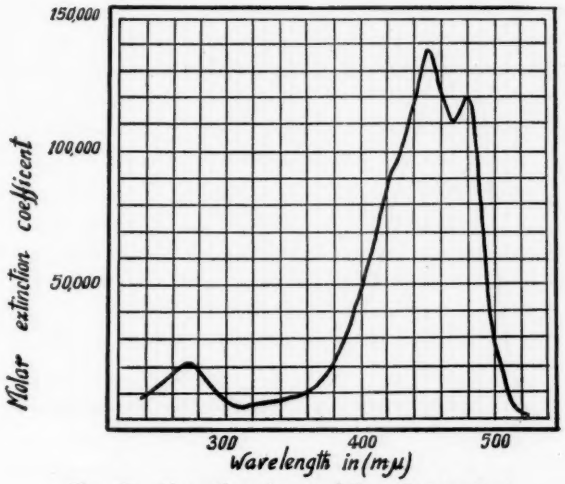


FIG. 2. Absorption characteristics of β-carotene.

Xanthophyll (also referred to in the chemical literature as lutein) has the formula $C_{40}H_{56}O_2$ and its structure which is shown in Fig. 3 exhibits its chemical nature as dihydroxy-α-carotene. The two end-groups in xanthophyll differ in their structure and this difference reflects the difference between α-carotene and β-carotene; the latter is symmetrical whereas the former is not. The characteristics of the absorption spectrum of xanthophyll in ethanol solution are exhibited by the spectrophotometer curve reproduced in Fig. 4. It will be seen that xanthophyll resembles β-carotene in its spectroscopic behaviour; but there are significant differences. In particular, the inflexion which appears in the absorption curve of β-carotene shows up as a distinct maximum at the same point in the case of xanthophyll.

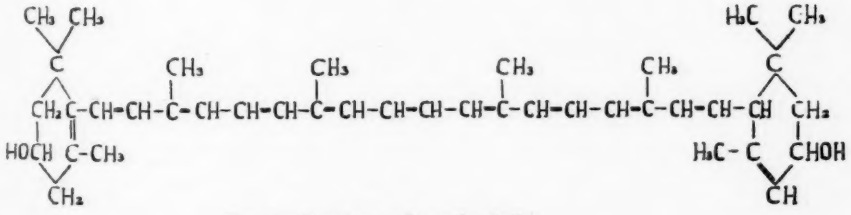


FIG. 3. Structure of xanthophyll.

The absorption spectra of the carotenoid pigments have been explained as arising from a swinging to and fro of the electrons along the length of their molecules. The incident radiation would excite such an oscillation provided that its electric vector is parallel to the length of the molecule. Simultaneously with the electronic oscillation and as an accompaniment to it, molecular vibrations would also be excited. The energy required for exciting the latter would add up to that of the electronic excitation. Hence the absorption spectrum would spread towards higher frequencies and smaller wavelengths. Peaks of absorption representing the successive harmonics of the most strongly excited molecular vibration frequencies may be expected to appear. It is thus possible to understand, at least in a qualitative fashion the characteristic features of the absorption curve: firstly, a steep rise at the long-wave end leading up to the first and fairly high maximum; after the first maximum, a few additional maxima and then a slow drop-down to a very weak absorption at the violet end of the spectrum.

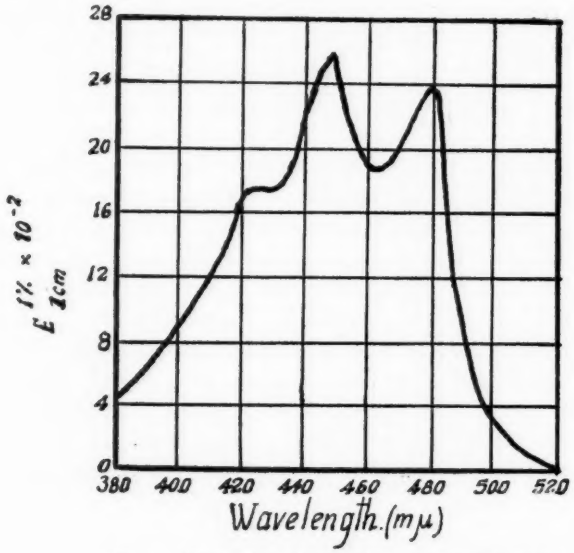


FIG. 4. Absorption characteristics of xanthophyll.

3. XANTHOPHYLL AND THE SPECTRAL COLOUR SEQUENCE

The colour of the spectrum to any observer with normal colour vision is green at 520 mμ, blue at 480 mμ and violet at 420 mμ. Referring to Fig. 4, it will be seen that the absorptive power of xanthophyll is zero at

520 mμ, has reached its first maximum at 480 mμ and that at 420 mμ it has passed its third maximum and is on its downward course. If xanthophyll is the visual pigment which functions in the wavelength range between 4000 and 5000 angstroms, the change-over from green to blue should appear in the vicinity of the steeply rising part of the absorption curve at about 490 mμ. This, indeed, is what is actually observed; the change of colour with wavelength is shown by the investigations already reviewed in the first part of this memoir to be very rapid in the vicinity of that wavelength. Similarly, one would expect the change from blue to violet to occur in the vicinity of the steeply falling part of the absorption curve around 440 mμ. At this wavelength, again, it has been observed that the colour changes rapidly with alteration of wavelength. In other words, the absorption-curve of the visual pigment and the colour sequence in the spectrum exhibit a close correlation, as indeed is to be expected since it is the visual pigment which by its absorptive properties enables the radiation to be perceived.

Fig. 5 reproduces the hue discrimination curve in the spectrum which appears on page 229 in the late Dr. P. J. Bouma's posthumously published book entitled *Physical Aspects of Colour*. It is stated that the curve represents the average of the results of a dozen different investigators whose published papers are listed in the book. (Some of these references are not available for consultation at Bangalore.) The graph shows a small dip at 420 mμ besides the more conspicuous ones at 490 mμ and 440 mμ, mentioned above. The dip at 420 mμ appears in the same position as the steep fall in the absorption curve of xanthophyll which commences after it has reached and passed its third maximum.

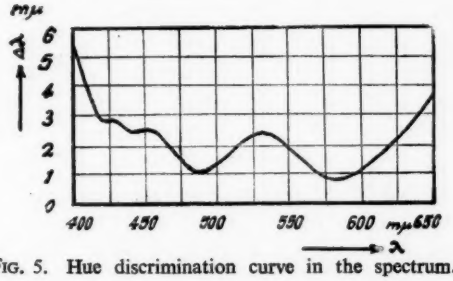


FIG. 5. Hue discrimination curve in the spectrum.

Besides the features referred to above, the blue-violet region of the spectrum exhibits other observable characteristics which should be mentioned and commented on here. The luminous efficiency which at 500 mμ is very much less than the maximum appearing in the greenish-yellow part of the spectrum shows a further rapid fall as we proceed towards shorter

wavelengths. This feature is represented in Fig. 6, the observations being those made by Jainski using a flicker method and a retinal illumination high enough to ensure that the results refer to photopic conditions. His observations do not take us beyond 460 mμ. But the simplest visual observations suffice to show that the luminosity of the spectrum becomes very small as we approach further towards its violet end. The other characteristic of the spectrum to which attention may be drawn appears in Fig. 5 reproduced above. The minimum of wavelength difference perceptible as a colour change exhibits a progressive increase as we approach the violet end of the spectrum, being some five times greater at 400 mμ than it is at 500 mμ. This increase accompanies and is superposed upon the undulations in the value of the minimum which appear in the same range of the spectrum. These features of visual experience are clearly related to the absorption characteristics of xanthophyll; the fall of luminous efficiency goes hand in hand with the decrease in absorptive power and the decreasing power of hue discrimination with the diminishing slope of the absorption curve, as we pass from the blue to the violet and approach the end of the visible spectrum.

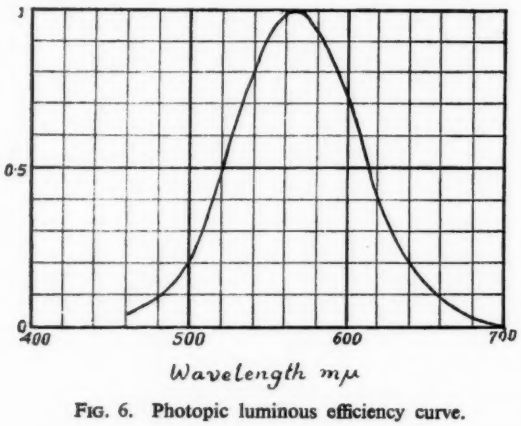


FIG. 6. Photopic luminous efficiency curve.

It appears worthwhile also to mention here that very simple visual observations with a pocket spectroscope and a moderately strong solution of xanthophyll prepared in the manner already stated suffice to establish the principal features in its absorption spectrum. With an absorption cell of small thickness, say 1 cm., one observes an enfeebled transmission in the blue and violet and a succession of absorption maxima in that region. This region exhibits a distinct edge at about 490 mμ beyond which there is

free transmission of light. A bluish-green section is visible which precedes the green and the rest of the spectrum. With a greater absorption path, say 3 cm., there is a complete cut-off of the violet and blue regions of the spectrum and a distinct enfeeblement of the bluish-green region. With a large absorption path, say 10 cm., the bluish-green section is also completely cut off, and we observe an absorption edge located at 510 mμ which separates the fully absorbed from the freely transmitted parts of the spectrum.

4. Effects Observed with Polarised Light

We have already had occasion to notice two very striking properties of xanthophyll, *viz.*, the elongated form of its molecules and the restriction placed on their power to absorb light and therefore also on the power of xanthophyll to function as a visual pigment, *viz.*, that the radiation falling on the molecules should have its electric vibration—or at least a component of it—parallel to the length of the molecules. These properties would have no observable consequences if the molecules of xanthophyll were orientated at random in the retina. This, however, is not the case in the foveal region which exhibits a depression or pit with the foveola at its centre and with sloping sides having a maximum slope angle of about 20°. As a consequence, the nerve fibres in the retina are normal to its surface only at the centre of the pit or depression. Elsewhere they slope away from the normal and as a result, the region of the fovea presents a radially disposed fibrous structure. The xanthophyll molecules are themselves highly elongated bodies and as they are part of the retina, they would naturally set themselves parallel to the elements of the fibrous structure in which they are located. In other words, the visual pigment would present to the incident light a radially disposed array of molecules with their long axes pointing towards the centre of the fovea. This radial disposition would be least evident at the centre of the fovea, would be most conspicuous in the region around the centre where the nerve fibres slope outwards and would cease again to be noticeable outside the foveal region.

If the observer views an extended source of unpolarised light, the special features of the foveal region referred to would not result in anything noticeable. With polarised light, however, the situation would be different. The molecules of xanthophyll which are parallel to the direction of optical vibration would function as absorbers and give rise to the sensation of a bright brush of light along that direction. The molecules perpendicular to the direction of vibration would be unable to absorb the light and hence a dark brush would appear along that direction. In other words, the observer would see an image of his fovea projected in space which presents the aspect

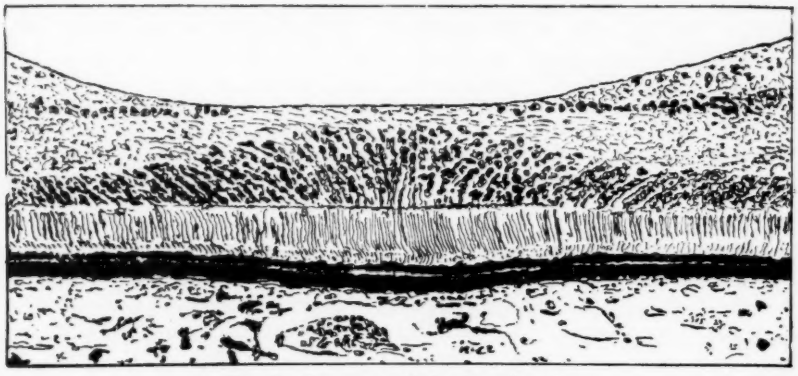


FIG. 7. Structure of the foveal region.

of a cross, the arm parallel to the direction of vibration in the incident light being bright and the arm perpendicular to it being dark.

Since the absorption of xanthophyll covers only the violet and blue regions of the spectrum but does not extend to greater wavelengths, it follows that the foveal cross seen in polarised light would be at its best if the incident light lies within those spectral regions. Light of greater wavelengths would not exhibit the phenomenon and hence its presence would only add to the general illumination of the field and thereby make the foveal cross a much less conspicuous phenomenon than it really is. This remark is of special importance in view of the relatively low luminosity of the blue and violet regions in comparison with the rest of the spectrum.

Since xanthophyll is a photopic visual pigment, it follows that the foveal cross requires for its observation that the illumination of the field with polarised light is sufficiently strong to ensure photopic conditions for the spectral region under consideration. When the illumination is reduced and we pass over to scotopic conditions, the effect should weaken and ultimately cease to be observable.

It should also be remarked that the phenomenon of the foveal cross should be equally well or even better seen binocularly as monocularly. For, what is actually observed is a picture of the foveal region projected on the field of view and when both eyes are used, their foveal regions are in register, in other words, both are seen at the same point in space.

It also deserves to be emphasised that the foveal cross seen in polarised light and the picture of the fovea seen with colour filters of various sorts

described in the second part of this memoir are closely related phenomena. In both cases we are concerned with a localised excitation of the visual receptors and the possibility of directly perceiving the results of such excitation. The closeness of the analogy will become plainer when we presently take up a description of the methods of observation and the results obtained.

5. Observation of the Effects

The phenomenon originally discovered by Haidinger, *viz.*, a faint brush which enables the eye to recognize polarised light and ascertain its plane of vibration is ordinarily both inconspicuous and fugitive in character. It however becomes a striking and conspicuous effect in the following circumstances: the field viewed by the observer should be of adequate intensity, *e.g.*, a cumulus cloud lit by sunlight or a brilliantly illuminated white screen; the observer should hold before his eyes (in addition to a polaroid) a colour filter which is transparent to the blue and violet rays and completely cuts out the rest of the spectrum; the polaroid should be rotated, or else oscillated continuously instead of remaining in a fixed orientation. With these arrangements, one observes a dark brush and crossing it transversely, a bright brush; the dark brush is very dark and the bright brush is brighter than the surrounding field. The two brushes together form the figure of a cross which fills an area which is readily recognizable as a projection in space of the fovea centralis of the observer's own retina. As the polariser is turned round or oscillated, the cross turns round or oscillates synchronously with it around a point which is the projection in space of the foveola of the observer's retina. If the polaroid and colour filter are both large enough, the whole phenomenon can be viewed binocularly.

The importance of using a colour filter which transmits the blue and violet regions of the spectrum and excludes the rest becomes clear when the observer substitutes for it a complementary filter, *viz.*, a yellow glass plate which cuts off the blue and violet regions and transmits freely the rest of the spectrum. *Not a trace of the foveal cross can then be seen.* It follows that the cross owes its origin to a visual pigment whose absorption appears in the violet and blue regions but does not extend to the rest of the spectrum. A more detailed examination may be made in different ways. One can, for example, view through the polaroid a field illuminated by a monochromator and alter the wavelength progressively. Alternatively, we may use the monochromatic radiations of the mercury arc; the brushes are fairly well seen with λ 4538 and also, but not so well, with the λ 4046 radiations. A simple method by which the entire spectrum may be scanned is to view the first order spectrum of a linear source of white light produced by a glass

diffraction grating held along with a polaroid in front of the observer's eye. The foveal cross can then be seen, if the observer directs his vision to the blue or violet region of the spectrum. But they are not visible in other parts of the spectrum, the transition from invisibility to visibility occurring rather abruptly around 4900 angstroms. The cross is clearest in the wavelength region between 4800 and 4300 angstroms. It is less distinct at still shorter wavelengths but nevertheless continues to be visible to the violet end of the spectrum.

A technique of observation different from that described above yields highly interesting results. With the colour filter held before his eye, the observer suddenly interposes a polariser in front of it. The cross then comes into view and slowly fades away. After a little while, the observer suddenly removes the polaroid. The cross is then seen once again but rotated through a right angle, the dark arm appearing in the place of the bright arm and *vice-versa*. On putting the polariser back, the cross regains its original configuration; and when the polariser is taken out once again, the cross turns round once again. It is thus clear that a sudden removal of the polaroid produces an effect of the same nature as that of turning it round through a right angle. This effect is clearly analogous to the phenomena observed with colour filters described in the second part of this memoir. The sudden removal of the polaroid results in the component vibration cut off by it being restored and allowed to fall upon the retina. As a consequence, the foveal cross is seen again but turned round through a right angle.

A convenient technique for studying the effects due to polarised light at different levels of illumination is to use a powerful and completely enclosed source of light, *e.g.*, a mercury arc or a tungsten lamp and to isolate the effective part of its spectral radiation by a colour filter which covers an aperture placed close to the source. The light which issues from the aperture can be received on a translucent diffusing screen and the light emerging through the latter is viewed by the observer through a polaroid from an appropriate distance. If the arrangement is set up in a long darkened chamber, the brightness of the field under observation can be varied over a large range by the simple device of moving the diffusing screen from a position close to the aperture from which the light issues to another sufficiently far away. Observations made in this manner show that the foveal cross is visible only when the illumination which falls on the diffusing screen is strong enough to allow ordinary print to be read. If the illumination be diminished further so that print ceases to be readable, the cross becomes

indistinct. When the illumination is so feeble that a printed page appears to the eye as a mere blur, the cross ceases altogether to be visible. The disappearance of the foveal cross thus goes hand in hand with the disappearance of the visual acuity which is a characteristic of photopic vision. It is also observed that the field of view as seen through the polaroid shows a progressive change in colour from a brilliant blue to a pale blue as the illumination is diminished to the point at which the foveal cross ceases to be noticeable. This is a further indication that we have then moved out from the photopic to the scotopic level of illumination in the blue and violet regions of the spectrum.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part IV. Ferroheme and Ferriheme

By Sir C. V. Raman

(*Memoir No. 125 from the Raman Research Institute, Bangalore-6*)

Received November 9, 1960

1. Introduction

In the first part of this memoir, the basic facts concerning the perception of light and colour were reviewed and a mechanism of the functioning of the retina was suggested which explains them in a simple and intelligible fashion. Human vision is mediated by certain pigments present in the retina, these pigments acting as energy-receiving and energy-transferring agents; in other words, they absorb the quanta of radiational energy incident on the retina, but pass on the energy thus absorbed to the sensory mechanism, themselves returning to their original states. The second part of the memoir dealt with the problem of determining the number and nature of the visual pigments functioning in the manner indicated, the regions of the spectrum in which they respectively operate and the distribution of the pigments over the area of the retina. A method of observation was described which furnishes valuable information on these points. Considerations were also developed which pointed to xanthophyll, ferroheme and ferriheme as the three visual pigments with which we are concerned in photopic vision. In the third part of the memoir, evidence was presented which confirms and establishes that xanthophyll is the visual pigment which functions in the violet and blue regions of the spectrum.

In the present part of the memoir, we are concerned with the region of the spectrum between the wavelengths 5000 and 7000 angstroms. The facts of observation which concern us here and need interpretation are firstly, the distribution of luminosity in this region of the spectrum; secondly, the distribution of colour in it; and thirdly, a derivative property of the same, namely, the characteristics of the colour progression which find expression in the so-called hue discrimination curves determined by various observers. We shall consider these facts here in some detail and discuss their interpretation. The aim is to infer therefrom the spectroscopic behaviour

of the visual pigments and to compare the same with what is known regarding the absorption spectra of the heme pigments.

2. Luminosity, Colour and Hue Discrimination

The form of the luminous efficiency curve in the spectrum depends to a notable extent on the region of the retina used in its determination. In what follows, we shall make use of the data obtained by Walters and Wright under photopic conditions in which only the foveal region of the retina was employed. Their results are reproduced below in Fig. 1. It will be noticed that the luminous efficiency exhibits a well-defined maximum at 5600 angstroms on either side of which it descends steeply, but less rapidly so on the side of longer wavelengths than towards the shorter ones. This pronounced asymmetry of form evidently calls for explanation.

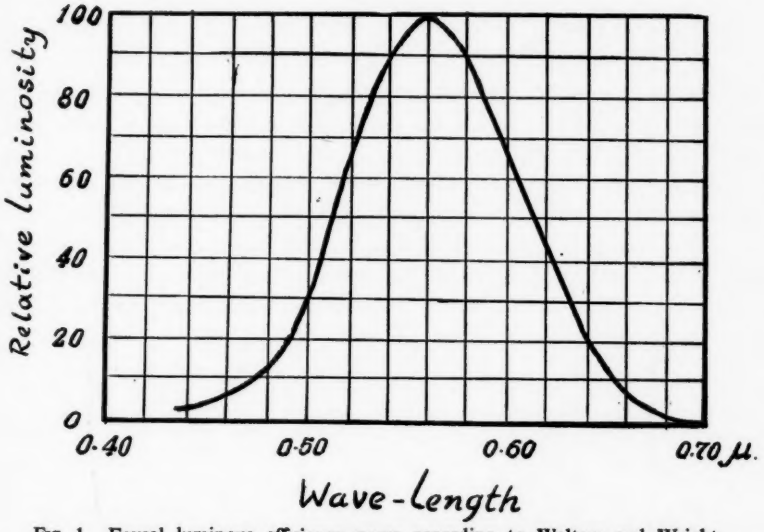
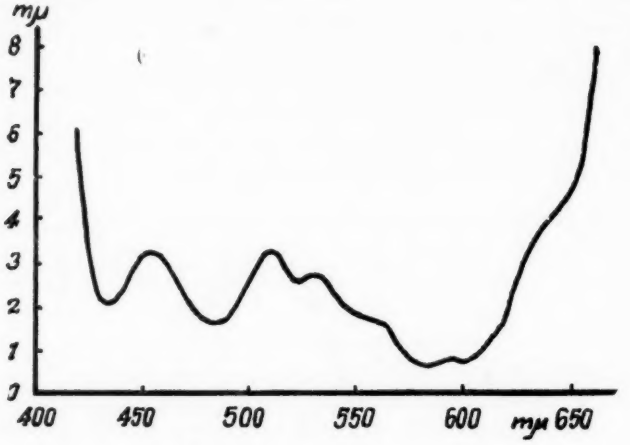


Fig. 1. Foveal luminous efficiency curve according to Walters and Wright.

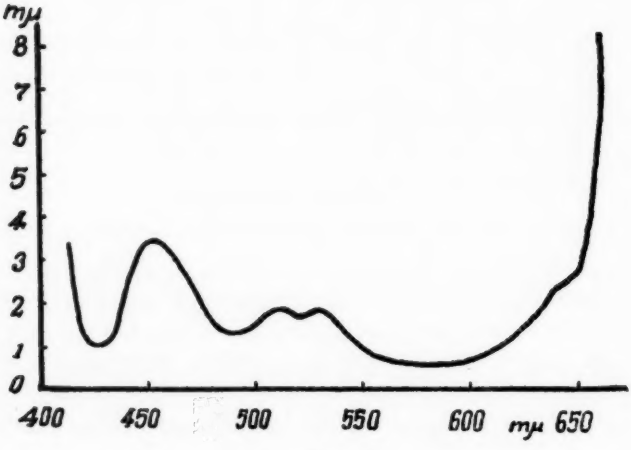
The graph of the luminous efficiency reproduced in Fig. 1 may be divided into four parts which exhibit distinctly different characters: (i) the foot of the graph from 4400 to 4950 angstroms where it exhibits a marked curvature; (ii) the steeply ascending part from 4950 to 5600 angstroms; (iii) the steeply descending part from 5600 to 6270 angstroms; (iv) the foot of the graph from 6270 to 7000 angstroms where again it exhibits a marked curvature.

On the basis of the average positions of the colour boundaries in the spectrum as placed by observers with normal vision, we may divide the

visible spectrum into four sectors thus: Blue-violet, $\lambda < 495$ mμ; Green, 495 m$\mu < \lambda < 566$ mμ; Orange-yellow, 566 m$\mu < \lambda < 627$ mμ; and Red, 627 m$\mu < \lambda$. It will be noticed that these sectors represent also the divisions of the luminous efficiency curve indicated above, except that the green-yellow boundary is placed at 566 mμ instead of 560 mμ where the maximum luminous efficiency appears.



(*a*)



(*b*)

FIG. 2. Hue discrimination in the spectrum (after Haase).

Measurements of the smallest change of wavelength needed for a perceptible change of colour have been made and reported by numerous observers. Their results agree in respect of the major features but show some differences in detail. This is not surprising, since the characteristics of human vision are by no means the same for all observers, and the techniques and physical conditions of observation are also not identical in all the investigations. Two slightly different forms of these hue discrimination curves have already been reproduced in earlier parts of this memoir. Figure 2 (*a*) and Fig. 2 (*b*) above represent the results reported by Haase for two different levels of illumination, Fig. 2 (*b*) representing the results for the higher level of the two which was ten times greater in intensity than the other. Figure 3 below reproduces the results of E.P.T. Tyndall for the wavelength range between 450 mμ and 650 mμ. The individual observations made by him at various times are marked in the graph by crosses and circles and show a remarkable consistency except in the vicinity of the humps at 530 mμ and 630 mμ respectively.

Tyndall's observations place the wavelength of minimum limen at 575 mμ where it has the value of 0·5 mμ; at greater wavelengths it increases, at first quite slowly and then more and more rapidly, reaching large values at the red end of the spectrum. The results reported by Haase and shown in Figs. 2 (*a*) and 2 (*b*) exhibit generally similar features in the same region. At the wavelengths around 530 mμ where Tyndall's data exhibit a considerable scatter, Haase's results show two minor humps instead of the single hump at 535 mμ reported by others.

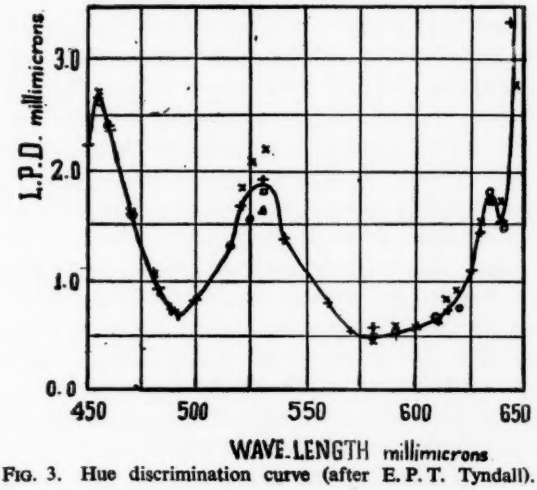


FIG. 3. Hue discrimination curve (after E. P. T. Tyndall).

3. THE COLOUR SEQUENCE IN THE SPECTRUM

The luminous efficiency curve reproduced in Fig. 1 above represents a summation of the effects of the three visual pigments functioning in their respective regions of the spectrum. It gives no indication of any special features related to those appearing in the hue discrimination curve at various points in the spectrum. We have therefore to assume that any special features appearing in the absorption curves of the individual pigments which determine their respective luminous efficiencies have been smoothed out and rendered unobservable by reason of such summation. However, since the econd visual pigment which functions in the green sector of the spectrum plays the major role in human vision, we are justified in assuming that the pronounced maximum at 560 mμ exhibited by the luminous efficiency curve arises principally by reason of a very conspicuous maximum at or near the same wavelength in the absorption spectrum of that pigment. On the other hand, in the blue-violet region of the spectrum, the first visual pigment which functions in that region would principally be responsible for the observed luminous efficiency. Likewise, the third visual pigment would be responsible for the observed luminosity in the region of longer wavelengths and its extension in that direction would account for the asymmetric form of the luminous efficiency curve.

The experiments and observations described in the second part of this memoir showed very clearly that there is a considerable overlap in the absorption spectra of the second and third visual pigments. That there is such an overlap and that it has a most important effect on the visual sensations excited by light appearing in the regions of such overlap is the clue to an understanding of the facts set forth above regarding the distribution of colour in the region of the spectrum now under consideration. The luminous efficiency curve itself indicates that there is a large drop in the absorptive power of the second visual pigment at wavelengths greater than 560 mμ and we may safely assume that this drop continues over the whole range in which that curve goes steeply down, in other words, up to about 627 mμ. The colour change from green to yellow, from yellow to orange and then from orange to red appears precisely in this region. It is a justifiable inference that these changes are a consequence of a rapid falling off in the luminous effect due to the second visual pigment and its progressive replacement by the luminous effect of the third visual pigment which, though inherently much weaker than that of the second pigment, nevertheless effectively determines the observed visual sensations in the regions of the spectrum where it is relatively more important. Continuing this line of

argument, we infer that when, at about 627 mμ, the colour of the spectrum passes over from orange to red, the second visual pigment has ceased to be effective, in other words, that its absorption strength is practically zero. On the other hand, the luminous efficiency curve itself indicates that the visual effect due to the third pigment persists up to the extreme red end of the spectrum, slowly and progressively decreasing with increasing wavelength.

4. Hue Discrimination in the Spectrum

The mechanism of the perception of light and colour by the eye which was indicated in the first part of the memoir was based on the quantum theory of radiation. It recognizes that the physical basis for the differences in colour perceived by the eye in the different parts of the spectrum is the fact that the magnitudes of the light-quanta differ from one end of the visible spectrum to the other, being least at the red end and greatest at the violet. To account for the remarkably high power of discrimination of colour actually exhibited by our eyes, it was postulated that the visual pigments in the retina which are the mediators of vision absorb the energy quanta incident on them and immediately pass on the energy thus absorbed to the sensory mechanism and return to their original energy states. Accepting this hypothesis, the following two questions arise which require an answer: What is the factor which limits the power of the eye to discriminate differences in colour in different parts of the spectrum? Why is it as great as it is in some parts, and why is it less in others? We shall now endeavour to find answers to these questions.

As is well known, the absorption of light by molecules embedded in solid or liquid media is manifested in the form of diffuse spectral bands exhibiting only remnants of the structure shown by their spectra in the state of vapour. As a typical example, we may mention the absorption spectrum of toluene in hexane solution observed in the near ultra-violet. Some nineteen bands are indeed discernible in the region between 270 mμ and 240 mμ, but only the first few of them are sharp and intense, and as we proceed further into the ultra-violet, they become weaker and more diffuse, and the individual bands can only with difficulty be distinguished apart from each other.

The absorption of light by the molecules of the visual pigments embedded in the retina and the transfer of the absorbed energy to the sensory mechanism would necessarily be influenced by various factors, including especially the thermal agitation in the medium. The absorption of radiation involves the electronic energy levels of the molecules of the pigment and the vibrational levels coupled with them. Thermal agitation, on the other hand,

appears as the energy of translatory movements. In solid and liquid media, the molecules may be regarded as being continuously in a state of collision, and hence exchanges of translatory energy *may* occur simultaneously with the exchanges of electronic and vibrational energy. Whether this happens at all and the extent to which it occurs may be expected to depend on the circumstances of each particular case. To obtain a rough idea of the effect of such exchanges, we proceed on the basis of the highly simplified picture of the process indicated by the following equation

$$h\nu - h\nu^* \approx \pm kT$$

in which h, k, and T are respectively Planck's constant, Boltzmann's constant and the absolute temperature of the retina, while $h\nu$ is the energy of the light incident on it and duly absorbed and $h\nu^*$ is the energy actually transferred to the sensory mechanism. The plus and minus signs refer to the cases in which the energy transferred is respectively diminished and increased by the presence of thermal agitation.
Taking

$$h = 6{\cdot}62 \times 10^{-27} \text{ erg.sec.}$$
$$k = 1{\cdot}38 \times 10^{-16} \text{ erg.deg.}^{-1}$$
$$T = 310°$$

$(\nu - \nu^*)$ when expressed as wave-numbers comes out as ± 215. In other words, the precision with which the eye can recognize a variation in the magnitude of the light quantum as a variation in colour in the spectrum would be diminished to the extent of 430 wave-numbers. When expressed as a wavelength spread in millimicrons, it comes out as directly proportional to the square of the wave-length in the spectrum, in other words, some four times greater at the red end of the spectrum than at the violet end. The calculated figures are shown in Table I below.

TABLE I

Effect of thermal agitation on hue discrimination

Spectral region in mμ	750	700	650	600	550	500	450	400
Spectral spread in mμ	24	20	18	16	12	10	8	6

Comparing the figures shown in Table I with the actual facts of observation as reported by various investigators, it is immediately obvious that except at the extreme red and extreme violet ends of the spectrum, the eye actually exhibits a power to detect changes of colour with change of wavelength far in excess of that indicated by the calculations appearing in Table I. Especially in certain parts of the spectrum, *viz.*, around 440 mμ, 490 mμ and 590 mμ, one finds a remarkable and obviously highly significant sensitivity to colour change with wavelength which closely approaches the theoretical perfection indicated by the principles of the quantum theory when the effect of thermal agitation is ignored. The inference is obvious, *viz.*, that the manner of calculation adopted greatly overestimates the influence of thermal agitation on hue discrimination. The transference of radiational energy to the sensory mechanism which takes place at the retina through the medium of the absorbing pigments present in it is presumably a very rapid process, and it is by no means inevitable that it would be influenced by the thermal agitation in the medium in the manner and to the extent contemplated in the calculations.

We are thus compelled by the facts to approach the subject of the varying power of hue discrimination in the spectrum from a different standpoint. We may reasonably expect it to be closely related to specific features in the absorption spectra of the visual pigments functioning in the respective regions of the visible spectrum; these features are not necessarily observable in the luminous efficiency curve, being masked by reason of the superposition of the effects of all three pigments in it. In the hue discrimination curve, on the other hand, they may well be expected to manifest themselves. In other words, the form of the hue discrimination curve is an indicator of the features of the absorption spectra of the individual pigments and is thus of prime importance in relation to the physiology of vision.

Already, in the third part of this memoir, it has been shown that the form of the hue discrimination curve in the blue-violet sector of the spectrum stands in the closest relationship to the special features in the absorption spectrum of the visual pigment functioning in that sector, *viz.*, xanthophyll. The minima of the limen of wavelength change for a perceptible alteration in colour appearing around 490 mμ and 440 mμ were, in fact, explained as due to the steep ascent and descent appearing in the absorption spectrum of xanthophyll respectively at those wavelengths. It is evident that we have likewise to seek for the explanation of the very remarkable minimum of limen noticeable in the wavelength region around 590 mμ on a similar basis.

Earlier, it has been remarked that the drop of the luminous efficiency at wavelengths greater than that of its maximum at 560 mμ should be

ascribed to a steep drop in the absorptive power of the second visual pigment in that region. Indeed, the latter drop should evidently be even steeper than that of the luminous efficiency curve. For, in the latter curve, the effect of the third visual pigment is superposed and since the latter evidently diminishes with increasing wavelength in this region, the steepness in the drop of the luminous efficiency curve would be diminished by reason of such superposition. We are thus entirely justified in inferring that in the region of wavelengths greater than 560 mμ, the second visual pigment exhibits a very steep drop in absorption with the result that it is practically negligible at wavelengths greater than 625 mμ. Accordingly, in the region of wavelengths between 560 mμ and 625 mμ, we should expect the limen for colour discrimination to exhibit a large diminution and that it should reach its lowest value in the region of wavelengths somewhere between those limits where the absorption curve of the second visual pigment exhibits its steepest fall, *viz.*, around 590 mμ. This is what is actually observed to be the case. The two D lines of sodium appear in this vicinity, and it is a well-established experimental result that when these two lines are equalised in their intensity, they exhibit an observable difference in colour.

5. THE ABSORPTION SPECTRA OF THE HEME PIGMENTS

The considerations of a general nature which lead us to the identification of the two visual pigments functioning in the green and red sectors of the spectrum as ferroheme and ferriheme respectively have already been set out in the second part of this memoir. We shall, in what follows, review the known facts regarding the absorption of light by the heme pigments with a view to compare them with the behaviour of the retinal pigments as indicated by the facts of visual experience.

The iron-protoporphyrin complex known as heme appears associated with different proteins in biological material. The extraordinary versatility which heme exhibits in performing a variety of physiological functions is ascribable to its association with the appropriate proteins in the different circumstances. Extensive studies have appeared in the literature regarding the absorption spectra which it exhibits in various cases. Indeed, the identification of the different heme proteins in biochemical research is largely based on their spectroscopic behaviour. The absorption of light by the heme proteins owes its origin in the first instance to the special structure characteristic of the porphyrin group of compounds, *viz.*, the tetrapyrollic group containing a closed ring of eighteen bonds which are alternately single and double. The character of the absorption is however modified when a metal atom enters the porphyrin structure and finds its place at the centre

of the ring. Further modifications in the spectroscopic behaviour appear when the metal-porphyrin structure associates itself with other nitrogenous materials, including especially different proteins.

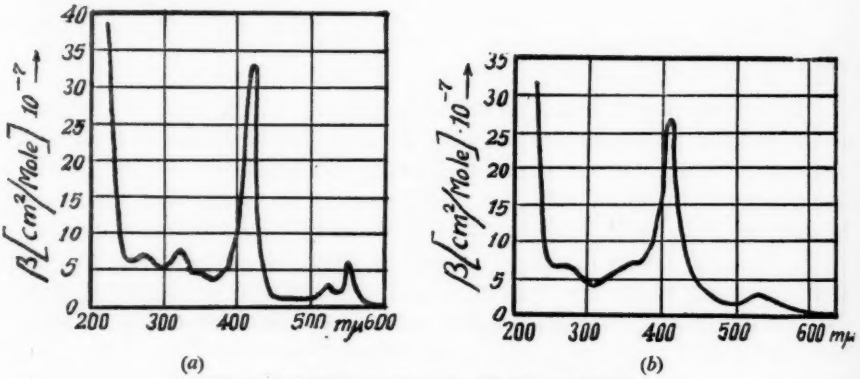
The porphyrins when dissolved in organic solvents exhibit a typical four-banded absorption in the visible spectrum with some indications of further details. The strength of the bands increases towards shorter wavelengths and in addition there is a still stronger band at about 400 mμ known as the Soret band, having been first observed by that author in the absorption spectrum of hemoglobin. The four bands of protoporphyrin, for example, in ether-acetic acid solution have their maxima at 632, 576, 537 and 502 mμ respectively, while indications of subsidiary bands at 605 and 585 mμ have also been noticed. It is worthy of remark that at the temperature of liquid air, the bands sharpen and are partly split up and shifted to shorter wavelengths.

The spectroscopic behaviour of the porphyrins in the form of complex salts formed by their combination with different metals has been extensively investigated, using the synthetically prepared substances in solution in different organic solvents. Generally speaking, it is found that the four-banded spectrum of the porphyrins is replaced by a two-banded spectrum, the position of the two maxima as well as their relative intensity varying with the metal which has entered the structure of the porphyrin. Ferrocoproporphyrin, for example, shows a strong absorption band at 550 mμ and a weak one at 520 mμ. The great difference between the spectroscopic behaviour of the complexes formed by the combination with iron in the ferrous and ferric states may be illustrated by the case of mesoporphyrin. Whereas the ferrous compound with it exhibits, in a buffered acetic acid solution, a band covering the region 555 mμ to 565 mμ in the green, the ferric compound shows two bands, one in the green between 530 mμ and 542 mμ and another in the red between 630 mμ and 640 mμ.

The association of the iron-porphyrin complexes with other nitrogenous substances to form what are known as hemochromes and hemichromes respectively has a notable influence on the character of their absorption spectra. The hemochrome structure is characterised by the appearance of a two-banded absorption spectrum of which the first or α-band is very sharp and also much more intense than the second or β-band of shorter wavelength. The positions of these two bands are observably influenced both by the nitrogenous base and by the porphyrin. In the hemochrome formed by protoporphyrin with pyridine for example, the α-band has its maximum at 558 mμ, while the β-band appears at 525 mμ.

On the other hand, the hemochrome formed by the combination of ferrous iron with mesoporphyrin and pyridine has its absorption maxima at 547 mμ and 518 mμ.

A reference should also be made here to the absorption spectra of the cytochromes, of which several have been reported and which are distinguished from each other by their spectroscopic behaviour. The best-known of them is cytochrome *c* which has been isolated and spectroscopically investigated in the pure state. It shows absorption maxima at 550 mμ and 521 mμ, the former or α-band being the more intense of the two. Figure 4 below reproduces the absorption characteristics of cytochrome *c* as determined by Theorell.



(*a*) (*b*)

FIG. 4. (*a*) Ferrocytochrome. (*b*) Ferricytochrome. Absorption spectra of cytochrome *c* after (Theorell).

Figure 4 (*a*) represents that of the reduced form, *viz.*, ferrocytochrome *c* and Fig. 4 (*b*) that of the oxidised form, *viz.*, ferricytochrome *c*. It will be noticed that while the absorption by the ferrous pigment exhibits a strong, sharp band located at 550 mμ and a weaker one at 521 mμ and is practically confined to the green region of the spectrum, the ferric compound has an absorption extending into the red with a broad diffuse maximum in the green around 530 mμ. This difference between the ferrous and ferric forms is further illustrated in Fig. 5 (due to Horecker and Kornberg) which shows that the absorption by the latter goes right up to the extreme red end of the spectrum.

6. THE OBSERVABLE CONSEQUENCES

If white light is spread out into a spectrum of small dispersion by a diffraction grating, the latter appears to the eye to consist of three bands

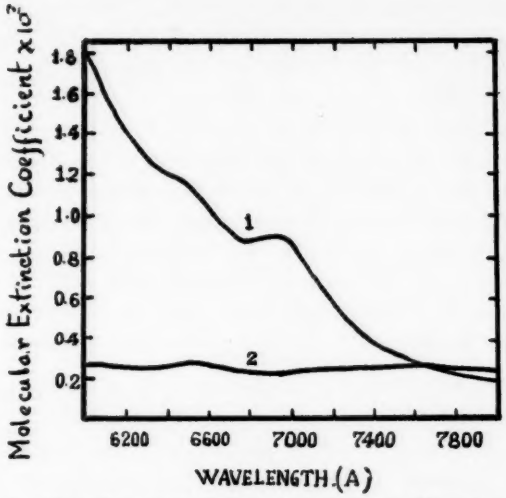


FIG. 5. Absorption spectrum of cytochrome *c* in the extreme red.
(1) Oxidised form; (2) Reduced form.

of colour of approximately equal width: a blue-violet band which is highly colourful but of low luminosity and covers the region from 400 mμ to 500 mμ; a green band from 500 mμ to 600 mμ and a red band from 600 mμ to 700 mμ. The two latter bands are also colourful, the red perhaps more so than the green, but the red is less luminous, especially near the extremity of the spectrum. The yellow-orange section of the spectrum appears merely as an edging between the green and the red. The most luminous part of the spectrum is in the green, not far from its yellow edge. The rapid changes in colour from blue to green at about 490 mμ, and from yellow to orange at about 590 mμ are also obvious to inspection. One is tempted to associate the three principal bands of colour with three visual pigments functioning in three distinct regions of the spectrum. But the appearance of the yellow and orange as a transition between green and red is a clear indication that there is an overlap of the absorption spectra of the second and third visual pigments.

The various related features of the spectrum mentioned above are readily intelligible in the light of the spectroscopic behaviour of the heme pigments discussed in the foregoing pages. The pigments formed by the combination of ferrous iron with protoporphyrin absorb light in the region of wavelengths between 500 mμ and 600 mμ. In all cases, the maximum of this absorption appears at or near 560 mμ which is also the wavelength

of maximum luminous efficiency in the spectrum. Then again, the absorption drops down quickly with increasing wave-length between 560 mμ and 600 mμ. This feature explains the low values for the limen of hue discrimination observed in that region. The absorption by the ferric forms of the pigment, on the other hand, appears both in the green and red sectors of the spectrum; it is, however, weaker and more diffuse than that of the ferrous forms. This situation explains the appearance of yellow and orange as transition colours between the green and the red in the region where the absorption by the second pigment is weak. Likewise, the diffuseness of the absorption by the ferric pigment and its progressive weakening with increasing wave-length explain the diminishing luminosity and the increasing limen of hue discrimination as we approach the red end of the spectrum.

Some remarks should be made here regarding the Soret band of absorption. Had this been effective as a mediator in human vision, the violet end instead of being the feeblest part of the spectrum, would have been the most luminous. The inference is obvious, that the Soret band is not active as a transmitter of the energy absorbed which, presumably, is dissipated in some other fashion.

The Soret band is a general feature in the absorption spectra of the whole porphyrin group of compounds, even when a metal atom is not present at the centre of the tetrapyrollic group. There is therefore no reason to believe that it would act in the same way as the absorption bands in the visible region appearing by reason of the presence of the metal atom. It may be remarked in this connection that chlorophyll participates in the photosynthetic activity of green leaves by reason of its characteristic absorption band in the red; the Soret band, so far as is known, remains entirely inactive.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part V. The Colour Triangle

By Sir C. V. Raman

(*Memoir No.* 125 *of the Raman Research Institute, Bangalore-6*)

Received November 24, 1960

1. Introduction

So far, in this memoir, we have concerned ourselves exclusively with the sensations excited by monochromatic radiations of different wavelengths appearing in the spectrum. The reason for this, as has already been explained in the first part of the memoir, is that only by such an approach is it possible to reach a correct understanding of the nature of the retinal processes which enable us to perceive light and colour. We shall now turn to the consideration of the more complex field which offers itself in the study of the sensations excited by heterogeneous light. Here again, the visual sensations resulting from monochromatic light necessarily form the starting-point of our approach to the subject. Indeed, the outstanding result which has emerged from all investigations in this field is the relation that all observed colours bear to the colours of monochromatic radiation. These latter stand in a category by themselves and form a kind of upper limit to the visual manifestations of colour.

The functioning of the three visual pigments present in the retina in their respective spectral regions will form the basis of our considerations. It will be shown that they enable a satisfactory elucidation to be given of the observed facts of the subject including especially those which in the past have been sought to be interpreted or explained in terms of the so-called trichromatic theory of vision. It is necessary here to emphasise that for a full understanding of the facts of heterochromatic vision, the role played by the central parts of the organ of sight is no less important than the functioning of the retina which is only the periphery of that organ. The function of the retina is to receive, absorb and pass on the energy of the incident radiation. But the synthesis which enables composite radiation consisting of energy quanta of different magnitudes to be perceived as a visual sensation can only take place in the central part of the visual organ. This is indeed

very clear from the facts of binocular vision. There is no colour sensation which can be produced by mixing two lights and presenting them to one eye which cannot be duplicated by supplying the two lights independently, one to each eye. As an example of this general principle, it will suffice to mention the familiar techniques employed in colour stereoscopy.

2. THE CHROMATIC SENSATIONS

As we proceed, it will emerge that the sensations which result from the superposition of radiations appearing in different parts of the spectrum fall into two categories which we shall term the *chromatic* and *achromatic* sensations respectively. We shall commence with a consideration of the chromatic sensations. The colours of the spectrum which represent the effect of monochromatic radiations on our visual organs are, of course, the chromatic sensations of the first order. In certain circumstances, however, the superposition of different monochromatic radiations may result in colour sensations which may be included in that category. We shall now consider these cases in order.

A group of cases of particular importance is that in which two radiations appearing respectively at the two ends of the spectrum, *viz.*, violet and red, are superposed. The observations described in the second part of this memoir show that the visual pigments which function at the two ends of the spectrum are *exclusively* the first and the third respectively. The energy-quanta at the red and violet ends of the spectrum also differ widely. There is no reason, therefore, to anticipate that the spectral components of the incident radiation would be confused with each other when the signals originating at the retina reach the cerebrum. Indeed, in this case, human vision very nearly succeeds in recognising the composite nature of the incident radiation. That the so-called purples are a mixture of red and violet is fairly obvious even to an inexperienced observer. The relative intensities of the two components make themselves felt in the hues perceived which form a complete sequence ranging from red at one end to violet at the other and rival the pure colours of the spectrum in their brilliance. It follows that the purples can be classed with the colours of monochromatic light as chromatic sensations of the first order. It is evident also that a mixture of two purples in any proportion would give us only another purple, in other words, nothing essentially different.

Another set of cases of special importance is that in which the two monochromatic radiations which are superposed both lie within the range of wavelengths between 530 mμ and 780 mμ. Xanthophyll which is the visual pigment functioning in the violet and blue sectors of the spectrum

does not absorb any light of wavelengths greater than 530 mμ. Hence, in the region between 530 mμ and 780 mμ, only two visual pigments, *viz.*, ferroheme and ferriheme function. The observations described in the second part of the memoir show clearly that there is a considerable overlap in their absorption spectra. It follows that when red and green radiations from the two ends of the range are superposed in any proportion, the resultant sensation would be one of the spectral colours falling within the same range. Indeed, two monochromatic radiations from anywhere between these wavelengths when superposed would reproduce a spectral colour lying elsewhere in the same range. These indeed are facts. They emerged quite clearly from Clerk Maxwell's investigations with his colour box and have been confirmed by all later investigations.

There is yet a third class of cases in which the superposition of monochromatic radiations gives rise to a chromatic sensation, *viz.*, those in which the superposed radiations are close to each other anywhere in the spectrum. They may be sufficiently far apart to be perceived as different in colour when viewed separately or in adjacent fields. Yet, when they are superposed, the eye fails to recognise the composite nature of the light and perceives a colour which may be described as the colour of a spectral frequency which is the weighted average of the frequencies of the superposed radiations, the weightage being determined by their respective luminosities.

3. The Achromatic Sensation

A spectroscopist would define white light as a stream of radiation which comprises energy-quanta of all possible values ranging over the entire visible spectrum and with an energy distribution such as would be found in the radiation from a black body at very high temperatures. Since, however, the central organ of vision is incapable of resolving the incident radiation into its spectral components, there is no reason for assuming that only such a radiation would be perceived by the eye as white light. Indeed, much less stringent requirements might suffice. We may remark here that the light falling on the retina is absorbed by three visual pigments which between them cover the entire range of the visible spectrum. Hence, the minimum requirement for the perception of the incident heterogeneous radiation as white light could well be the following: *all the three visual pigments should function and should contribute to the observed luminosity in the same proportions as they would if the incident radiation were white light in the spectroscopic sense.* We shall provisionally accept this requirement as adequate and compare its consequences with the actual facts of observation,

The green light appearing in the wavelength region between 495 mμ and 566 mμ stands in a category by itself. In this sector of the spectrum, vision is mediated almost exclusively by ferroheme though the other two visual pigments make sensible contributions respectively near the two ends of the sector. It follows that to achromatise green light, one would require the addition of radiations from both ends of the spectrum where xanthophyll and ferriheme respectively function. The complementaries to the green of the spectrum accordingly lie in the region of the purples; as we pass from the boundary between blue and green to the boundary between green and yellow, the location of the complementary colour would shift from the red to the violet end of the series of purples.

As has been remarked earlier, the yellow colour of the spectrum between 566 mμ and 589 mμ and the orange colour between 589 mμ and 627 mμ arise by reason of the circumstance that the absorption spectra of ferroheme and ferriheme overlap in these regions; in the yellow sector, their absorptions are of comparable strength, while in the orange, the third pigment is distinctly the more effective. As a consequence of this, the complementary colour to yellow would be at the violet end of the spectrum; as we move into the orange, the complementary colour would shift into the blue. A further shift towards the red would result in the complementary colour being located at the boundary between the green and blue sectors in the spectrum. The remarkable fact of observation that in a whole series of cases the superposition of only two monochromatic radiations with appropriate intensities results in a complete suppression of colour thus finds a simple and satisfactory elucidation on the basis of the present approach to colour theory.

4. SUPERPOSITION OF THE CHROMATIC AND ACHROMATIC SENSATIONS

We have seen that in certain cases, non-homogeneous light excites chromatic sensations identical with the colours of the spectrum or the purples derived therefrom, while in other cases the resulting sensation is achromatic. We may therefore assume that, in general, both of these effects would be manifested but to different extents depending on the particular circumstance of each case. In other words, the sensation excited by non-homogeneous light could, in general, be described as a superposition of the chromatic and achromatic sensations. The colours of the spectrum and the purples accordingly set an upper limit to the visual manifestations of colour. We infer that non-homogeneous light exhibits a third attribute besides luminosity and colour, namely, the purity or degree of saturation of the colour. The highest purity is that of the pure spectral colours and the purples derived therefrom, while the lowest purity represents the case

in which the achromatic part is relatively so large that no colour is discernible. Hand in hand with the concept of purity enters also the concept of dominant wavelength, which is the particular wavelength in the spectrum the colour of which the composite radiation under study most nearly resembles.

An interesting question arises here. Should the chromatic and achromatic sensations associated with non-homogeneous light be regarded as distinct effects or as inseparable from each other? If one thinks in physical terms, there is clearly a fundamental difference between them. An achromatic sensation would correspond to a chaotic and characterless disturbance; on the other hand, a pure spectral colour is associated with specifiable quanta of radiational energy. There is no reason why sensations so different in their nature and origin should be placed in the same category. It seems more appropriate to regard them as quite distinct attributes of the sensations excited by non-homogeneous light.

The very interesting results obtained by E.P.T. Tyndall and by G. Haase in their studies on colour discrimination with admixtures of monochromatic and white light have a bearing on the issue raised above. Measurements were made by these authors of the smallest change in wavelength of monochromatic light necessary to produce a detectable change of colour. The determinations were then repeated when white light was added in equal amounts to the two monochromatic fields of slightly different wavelength under comparison, the purity or degree of saturation of the colour in these fields being thus varied in different observations over a wide range. The remarkable result emerged that the chromatic sensibility of the eye to wavelength differences is not significantly diminished even when the white light added represents a fifty per cent. dilution of the visible colour. A result of this nature could scarcely have been anticipated unless the chromatic and achromatic sensations are distinct and unrelated effects.

6. The Results of Colour-Mixing Experiments

The simplest kind of experiment that could be made on the mixing of colours is to have only two monochromatic radiations, the spectral position and relative intensities of which could be varied, and to compare the sensation resulting from their superposition with another monochromatic radiation appearing in an intermediate position in the spectrum, the intensity of which can also be varied. The results of such comparison can be broadly indicated in the light of the remarks made above.

If both the selected radiations lie within the wavelength range between 530 mμ and 780 mμ, there would be little difficulty in obtaining a perfect

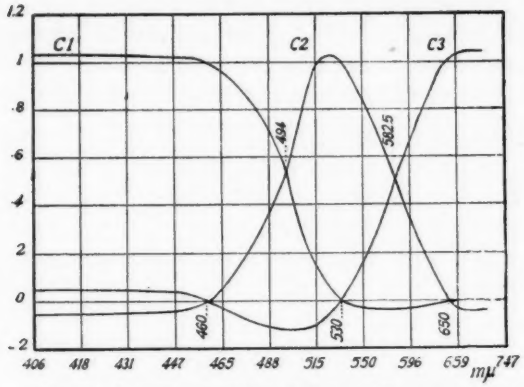


FIG. 1. Results of mixing 460 mμ, 530 mμ and 650 mμ.

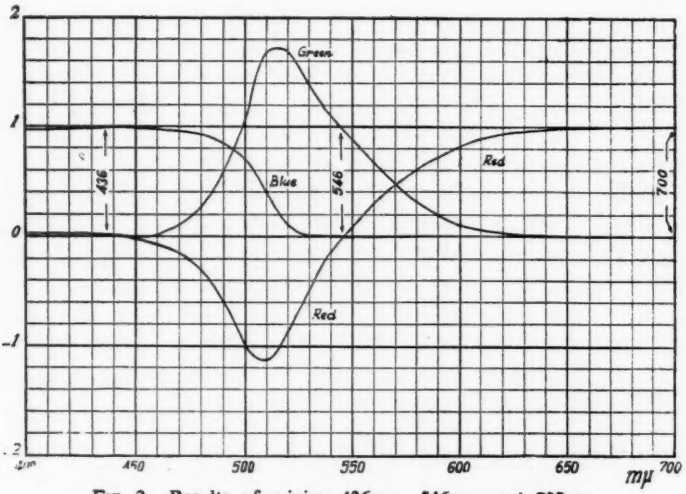


FIG. 2. Results of mixing 436 mμ, 546 mμ and 700 mμ.

match. Likewise, if one of the selected radiations is near the extreme red end or near the extreme violet end, and the other also lies in the violet or red sector of the spectrum as the case may be, there should be no difficulty in matching the result with some intermediate radiation. The situation would however be different if one of the selected radiations lies in the wave length range between 400 mμ and 530 mμ and the other also lies in that range, but not in an adjacent position. Only when the two selected radiations are quite close to each other that it would be possible to obtain a good

match. The further away they are, the less and less satisfactory would be the result, until finally when the two are sufficiently far apart, there could be no comparison at all. The position would be far worse if one of the radiations is in the wavelength range between 400 mμ and 530 mμ and the other is in the range between 530 mμ and 750 mμ. We would then be approaching a situation in which the result of mixing the two monochromatic colours would be to obtain an achromatic sensation.

Figure 1 and Fig. 2 above represent the results of experiments of the same nature as that indicated above with the difference that three instead of two monochromatic radiations were chosen and employed and while their positions in the spectrum were kept fixed, their intensities were varied with a view to obtain a match with the spectral colours appearing over the whole range of the spectrum. In Fig. 1, the three chosen wavelengths were 460 mμ, 530 mμ and 650 mμ and the results represented are those of W. D. Wright and collaborators. In Fig. 2, the chosen wavelengths were 436 mμ, 546 mμ and 700 mμ, the two former being the strong lines in the mercury arc spectrum. The graphs appearing in the figure represent the values of the coefficients C_1 (blue), C_2 (green) and C_3 (red) which indicate the quantities of blue, green and red light necessary to obtain the match represented by the colour equation

$$C_1B + C_2G + C_3R = \text{Chosen spectral colour},$$

where

$$C_1 + C_2 + C_3 = 1.$$

Figures 1 and 2 show certain features in common and also some noteworthy differences. We shall first mention the former and remark on their significance in relation to the absorptive properties of the visual pigments. In both figures, the coefficient C_1 (blue) has a value of nearly unity in the violet sector of the spectrum and then drops down steeply in the wavelength range 480 mμ to 530 mμ and is negligible or zero at all wavelengths greater than 530 mμ. The behaviour of C_1 thus clearly follows the absorption characteristics of xanthophyll. Then again, in both figures, the graphs for C_2 (green) and C_3 (red) overlap in the wavelength region between 550 mμ and 625 mμ; C_2 diminishes and C_3 increases in this range, the graphs crossing at 582 mμ in Fig. 1 and at 570 mμ in Fig. 2. C_2 becomes negligible in comparison with C_3 at all wavelengths greater than 625 mμ, while C_3 is dominant and practically unity in that region. Here, again, the behaviours of C_2 and C_3 recall the remarks made earlier regarding the overlapping of the absorption spectra of ferroheme and of ferriheme and its consequences.

The appearance of negative coefficients for C_3 in the spectral region between 460 mμ and 530 mμ is a well-marked feature in both figures but far more so in Fig. 2 than in Fig. 1, evidently because the blue and green radiations superposed were farther apart in the spectrum in the case of Fig. 2 than of Fig. 1. The appearance of these negative coefficients indicates that the superposition of the two monochromatic radiations results in a strong achromatic component in the sensation. A good measure of the third component has therefore to be added to the spectral colour under study to obtain a colour match. The production of an achromatic sensation by the superposition of monochromatic radiations in certain circumstances is thus an important and indeed basic feature in colour theory. The circumstances in which the achromatic sensation appears have already been discussed in Section 3 above and need not therefore be repeated here.

7. GEOMETRIC REPRESENTATIONS OF COLOUR

Figure 3 below reproduces the so-called XYZ chromaticity diagram. This represents in geometric form certain empirically determined colour relationships which have been put into a shape convenient for practical use.

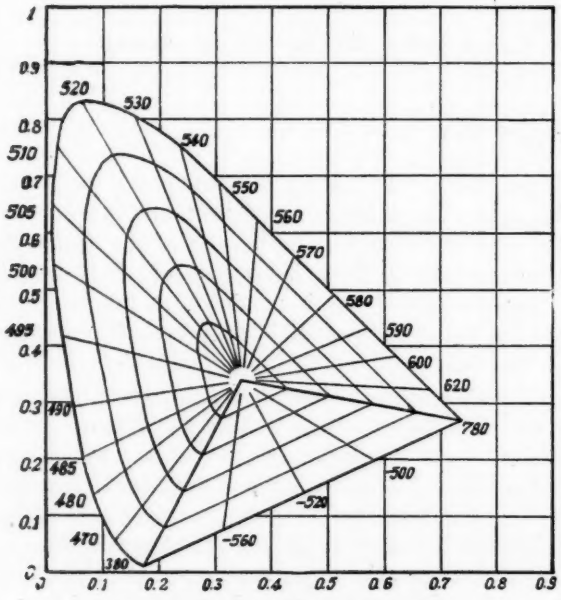


FIG. 3. The XYZ chromaticity diagram.

The diagram is reproduced here for the reason that the facts concerning colour vision elucidated in the preceding pages are evident on a simple inspection of it.

1. All observable colours appear as points inside a closed figure at the periphery of which appear the colours of the spectrum and the line of purples. The latter is a straight line joining the violet and red ends of the spectrum.

2. The spectral colours in the range between 530 mμ and 780 mμ appear on a line which is straight except very near 530 mμ where it exhibits a slight curvature.

3. Chromatic sensations complementary to each other are indicated by the two points on the periphery of the figure the straight line joining which passes through its white centre.

4. The degree of saturation or purity of any observed colour is indicated by its position in the figure on the line which joins the white centre with the point on the periphery representing the dominant wavelength.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part VI. Defective Colour Vision

By Sir C. V. Raman

(*Memoir No.* 125 *of the Raman Research Institute, Bangalore-6*)

Received December 1, 1960

1. Introduction

The subject of defects in colour vision is one of very general interest. The actual nature of the defects, the different types of defect and the relationships between them, the hereditary character of the defects, the frequency of their appearance in the human population and their distribution as between the sexes, the manner in which they can be brought to light and their relationship to occupational fitness are some of its different aspects. The scientific investigation of the characters of defective colour vision is a necessary preliminary to a deeper understanding of its nature and origin. In view of the many-sided nature of the subject, it has naturally been the theme of numerous studies and researches and an extensive literature has grown up dealing with it from different points of view.

In the present memoir, we shall concern ourselves chiefly with the fundamental aspects of defective colour vision. The subject will be dealt with on the basis of the ideas set forth and developed in the preceding parts of the memoir.

2. The Origin of the Defects

The defects of colour vision which we shall proceed to discuss in detail are of congenital origin and belong to the so-called sex-linked and recessive type of inheritable characters. The principles of genetics indicate, in agreement with observation, that defective colour vision should be far less common amongst women than amongst men; usually the woman acts simply as a carrier and transmits the defect without showing any characteristic anomaly herself. The fact that the defects are passed on from parents to progeny is of deep import and suggests that they stand in the closest relationship to the functioning of biochemical processes in the human body. In this connection, it is appropriate here to recall other inheritable characters

which are clearly of a biochemical nature. The blood group to which an individual belongs is one of them. Another is the rare condition known as hæmophilia, which is the failure of human blood to stop flowing out from a wound when a person has been accidentally injured. This is known to be a sex-linked recessive defect.

By far the commonest and most thoroughly studied defects in colour vision are those which relate to the perception of the light appearing in the region of the spectrum between 495 mμ and 780 mμ, in other words those covered by the green, yellow, orange and red sectors in the spectrum as perceived by a person with normal colour vision. In this region, as we have seen, vision is mediated by the two visual pigments ferroheme and ferriheme. These have similar structures which differ only in that the iron atom appearing at the central position of the tetrapyrollic group in one case is in the ferrous and in the other in the ferric state. The question naturally arises as to the nature of the biochemical mechanism which regulates the proportion in which the two pigments appear in the retina. The passage from the ferrous to the ferric state is a change in valency and may be regarded as an oxidative process. It is to be presumed that there is a mechanism at work by which the formation of the ferric pigment is permitted up to a certain proportion and its further progress beyond that point is inhibited.

If the suggestion made above represents the actual position, it follows that the mechanism might, at least in some cases, not function in the normal manner. For example, it is possible that the oxidation is totally inhibited, in which case only ferroheme would be present in the retina. Then again, there might be cases intermediate between such a complete inhibition and the normal functioning of the mechanism. It is also possible that there might be a lack of balance in the opposite direction and some cases in which the oxidation goes so far that ferriheme is in excess of that needed for normal colour vision.

In what follows, we shall consider the consequences of the biochemical situations indicated above and compare them with the actual facts elicited by studies of the different types of defective colour vision.

3. Protanopic and Protanamolous Vision

The cases of defective colour perception which came first under notice and received the largest share of attention were naturally those of the extreme kind which revealed themselves without any special efforts being made to discover them. Subsequently, however, scientific studies showed that the incidence of the defects is larger than was suspected and that there is a

considerable variation in the actual magnitude and character of the defects. At the present time, if we leave aside some rarer types, the kinds of colour vision which have been recognized and investigated are five in number: I. Protanopic vision (1%). II. Protanamolous vision (1%). III. Normal vision (92%). IV. Deuteranamolous vision (5%). V. Deuteranopic vision (1%). The approximate percentages of the male human population belonging to these classes have been entered in round figures after each of them. The first and the fifth kinds are usually grouped together as dichromatism, while the second and fourth are commonly referred to as anamolous trichromatism, in other words as variants of normal vision.

We shall first consider protanopic vision. Its characters may be deduced from those of normal colour vision discussed in detail in the fourth part of this memoir. In the absence of ferriheme, the distribution of luminosity in the spectrum, the colour sequence observed and the form of the hue discrimination curve would all be necessarily disturbed. The luminosity would be zero in the sector of the spectrum between 650 mμ and 780 mμ and would be diminished relatively to the rest in the regions of the spectrum where ferriheme, when present, contributes to the observed luminosity. The red sector would disappear completely from the spectrum. In normal vision, ferroheme and ferriheme co-operate in the regions where yellow and orange are seen, and hence in the absence of ferriheme these colours cannot be perceived. Protanopic vision which operates by the mediation of only two pigments, namely, xanthophyll and ferroheme, would accordingly present only two sectors in the spectrum, one on either side of the wavelength 495 mμ which forms the boundary between blue and green in the normal colour sequence. Hence, as in normal vision, that wavelength would continue to be the point in the spectrum where the colour as perceived by the protanope changes most rapidly. The features of the hue discrimination curve observed at greater wavelengths would, however, disappear and be replaced by a continuous falling off in the rate of change of observable colour with wavelength. The features of protanopic vision thus deduced are in agreement with those actually observed. The comparisons between normal and protanopic vision in respect of spectral luminosity and hue discrimination exhibited respectively in Figs. 1 and 2 are based on the observations of F.H.G. Pitt.

We now proceed to discuss the characteristics of protanamolous vision on the assumption that this type of vision results from a replacement of the ferroheme which alone is present in protanopic vision by a small proportion of ferriheme. The effect of such replacement on vision would be principally

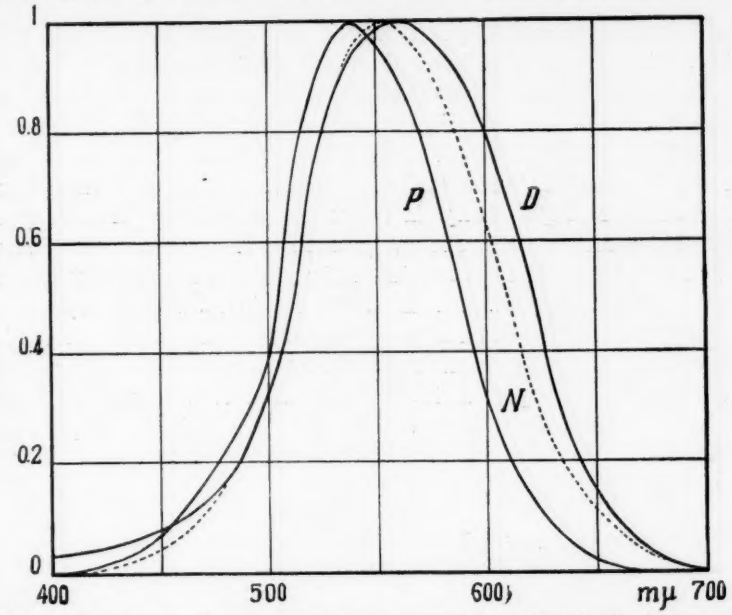


FIG. 1. Comparison of spectral luminosity curves for protanopic (P), deuteranopic (D) and normal vision (N).

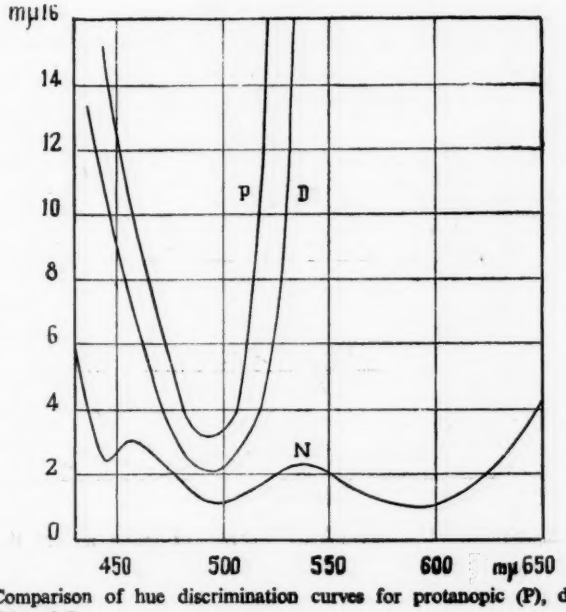


FIG. 2. Comparison of hue discrimination curves for protanopic (P), deuteranopic (D) and normal vision (N).

felt in the region of wavelengths where the absorption of ferriheme is comparable with or actually stronger than that of the ferroheme. This is the region between 560 mμ and 650 mμ where in normal colour vision, green changes over to yellow and then to orange and red. In this region, the protanamolous observer would see hues not perceived by the protanope, and recognise that they alter with the location in the spectrum. In other words, besides the rapid change in colour around 495 mμ which is apparent alike to the normal observer and the protanope, the protanamolous observer would observe colour changes roughly analogous to those apparent to normal vision in the region of wavelengths between 560 mμ and 650 mμ, but of a much less precisely defined character. The greater the quantity of ferriheme which replaces ferroheme, the more clearly would these features be perceived. It may therefore be expected that the protanamolous vision would exhibit in its hue discrimination curves a wide range of variation, approximating to normal colour vision at one end of the range to that of protanopic vision at the other end.

The conclusions reached above find support in the experimental data represented in Fig. 3 below of the hue discrimination curves of four pro-

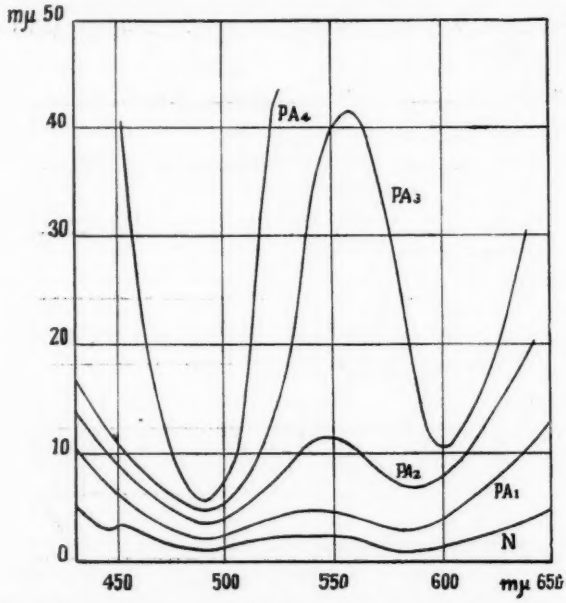


FIG. 3. Comparison of hue discrimination curves of protanamolous (Pa) and normal observers (N).

tanamolous observers and one normal observer which have been selected from the extensive set of data presented in a paper by McKeon and Wright.

4. Deuteranopic and Deuteranamolous Vision

A biochemical situation which results in the presence of an excess of ferriheme over ferroheme would have consequences in respect of colour vision which can readily be foreseen. As the ferroheme-ferriheme ratio diminishes, the region of wavelengths in the spectrum within which the absorptions of the two pigments are of comparable strength would shift towards shorter wavelengths and their overlap would extend and become effective until it completely covers the sector of the spectrum which appears green to a normal observer. In other words, the green and red sectors of the spectrum cease to have a separate existence and merge to form a tract in which the yellow and orange regions which ordinarily form only a fringe between them extend and cover the entire range of wavelengths referred to. Thus, instead of the normal colour sequence of green, yellow, orange and red, a band of colour would appear which may be described as yellow with a greenish tinge at one end and a reddish tinge at the other. This would cover the entire region where the absorption spectra of ferroheme and ferriheme co-operate. It would terminate at about 495 mμ where the blue-violet sector of the spectrum begins. Thus, again, as in protanopic vision, the spectrum consists of only two sectors meeting at about that wavelength. The difference between deuteranopic and protanopic vision is that in the former case, the spectrum goes up to the extreme red end instead of stopping off at shorter wavelengths. The colours observed would also be different in the two cases.

Observational evidence confirming the correctness of the foregoing explanation of deuteranopic colour vision is furnished by the spectral luminosity curves and the hue-discrimination data for several deuteranopic subjects which have been made available by the work of F. G. H. Pitt. These have been represented alongside of those of protanopic and normal observers in Figs. 1 and 2 above respectively in the text. They show very clearly the much greater extension of the spectral luminosity curves towards the red in deuteranopic as compared with protanopic vision. The difference between deuteranopic and normal vision in respect of the spectral luminosity curves is also distinctly shown. The hue discrimination curves, on the other hand, show a general similarity between protanopic and deuteranopic vision and a striking dissimilarity with the normal vision. The observations, however, reveal the noteworthy and significant fact that deuteranopic vision

has a distinctly better hue discrimination than protanopic vision in the wavelength range between 400 mμ and 530 mμ. But hue discrimination is completely lacking in the region of greater wavelengths in both types of vision.

We shall not here pause to discuss the results of colour mixing experiments made with observers having one or the other of these two types of defective vision, as also studies of the colour confusions which they exhibit when presented with test objects which appear of different hue to normal observers. These features of protanopic and deuteranopic vision are readily deducible from the fundamental results set forth above. We shall proceed to consider the features of deuteranamolous vision which are of special interest if only for the reason that this type of defective vision outnumbers all the other types put together. Deuteranamolous vision can be distinguished from normal vision by the method of observation by which its existence as well as that of protanamolous vision was discovered, *viz.*, that of requiring the person tested to match a monochromatic yellow by a mixture of monochromatic green and red radiations by varying their relative intensities. A protanamolous observer would require a smaller ratio of green to red than is required for normal vision, while a deuteranamolous observer would need a larger ratio. This is a clear indication that red light appears dimmer in protanamolous than in normal vision, while it appears brighter in deuteranamolous vision. Since protanomaly is explicable as a consequence of the ferroheme-ferriheme ratio being larger than the normal, it may be inferred that deuteranomaly is a result of ferriheme being present in the retina in excess of that required for normal colour vision.

The observable consequences of ferriheme being present in a proportion greater than normal should be of three kinds: (*a*) a shift of the spectral luminosity curves towards longer wavelengths as compared with normal; (*b*) a reduction in the power of hue discrimination at all wavelengths greater than 5000 Å and (*c*) changes of the chromatic coefficients, *viz.*, the intensity ratio between green and red monochromatic radiations of chosen wavelengths which when superposed would match the colours of the spectrum at various wavelengths. In respect of all these features, it may be expected that the magnitude of the observed changes would depend upon the actual proportion of ferriheme present. The more nearly the ferroheme-ferriheme ratio approaches normal, the greater would be the resemblance between deuteranamolous and normal vision. *Per contra*, the greater the excess of ferriheme present, the more would deuteranamolous vision tend to approach deuteranopic vision in its characters.

The observational evidence available supports the foregoing inferences. D. B. Judd in a published report has drawn the individual spectral luminosity

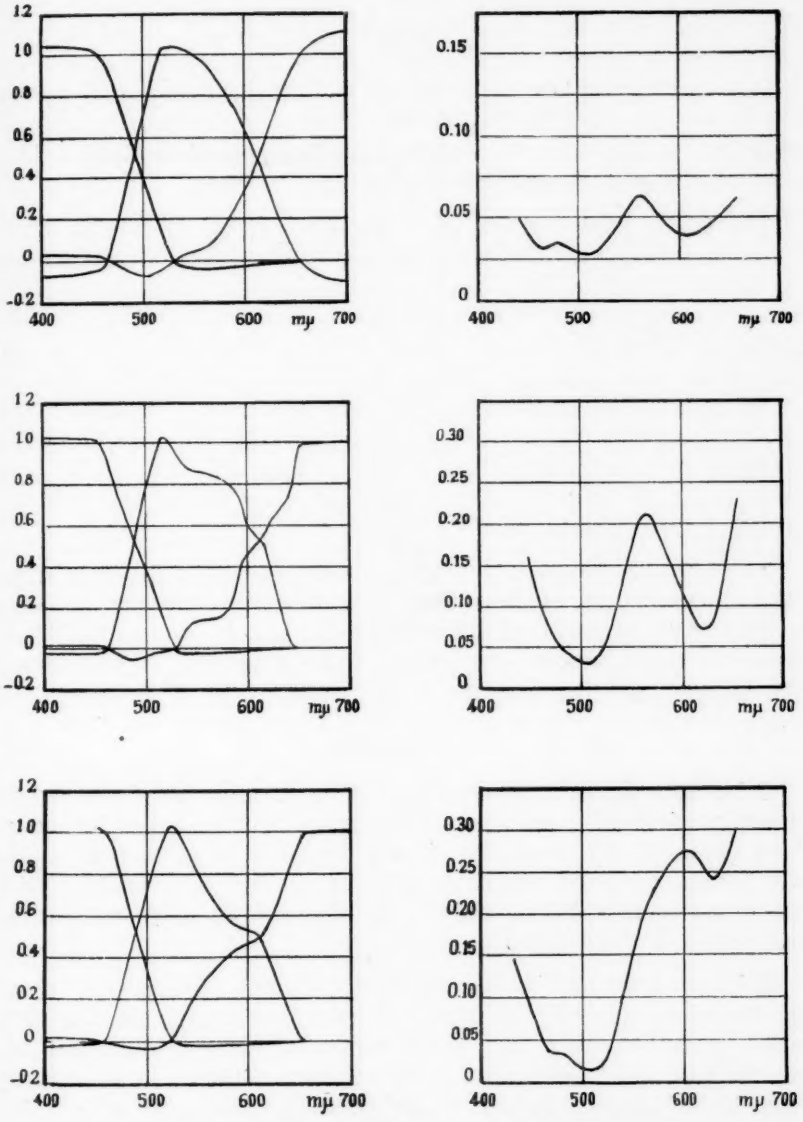


FIG. 4. (*a*) Spectral chromaticity coefficients and (*b*) hue discrimination curves of three deuteranamolous observers according to J. H. Nelson. The unit of the ordinates in Fig. 4 (*b*) is 100 mμ.

curves of twelve protanamolous and of six deuteranamolous observers and compared them respectively with the averages of the luminosity curves of six protanopes and of six deuteranopes. As is to be expected, the luminosity curves of both the protanamolous and of deuteranamolous observers exhibit a certain spread amongst themselves. The significant result emerges that the average curve for the six deuteranopes appears clearly separated and shifted towards longer wavelengths from the region where the individual curves of the deuteranamolous observers are recorded.

The fall in power of hue discrimination and the alterations of the chromatic coefficients are necessarily related to each other. For, the extension of the overlap of the absorption spectra of ferroheme and ferriheme due to the latter being present in excess would obliterate the colour difference between the green and the red sectors of the spectrum. This, on the one hand, would result in a large increase in the limen for hue discrimination and also shift the wavelength where the minimum of limen appears towards the red where there is no such overlap. Simultaneously, the chromatic coefficients for green and red would tend to approach and become equal to each other in the same region where the power of hue discrimination registers a large diminution.

The foregoing inferences find very clear support in the data represented in Fig. 4 where the chromatic coefficients and the hue discrimination curves for three different deuteranamolous observers are shown side by side to exhibit the correlations between them. These have been selected from a more extensive set of observational data published by J. H. Nelson.

5. TRITANOPIA

It has been shown in the third part of this memoir that the carotenoid pigment xanthophyll is the visual receptor in the blue and violet regions of the spectrum. Its absorption which is negligible at wavelengths greater than 520 mμ, rises very steeply around 490 mμ and after reaching fairly high values between 480 mμ and 440 mμ drops down again to relatively small values in the far violet.

It follows from what has been stated that if xanthophyll is totally absent from the retina, colour vision would be seriously affected in the blue-violet sector. In the first place, the observer would be unable to distinguish between the blue and green colours in the spectrum. The luminous efficiency would fall off towards the end of the spectrum rather more rapidly than it would for a normal observer. The hue discrimination curve should also show striking abnormalities. While the usual well-marked minimum of

the limen around 590 mμ would continue to be observed, the form of the curve in the green and blue regions would be totally different. The maximum value of the limen observed around 540 mμ and the minimum around 490 mμ would both disappear. Instead, there should be a progressive increase in the limen with diminishing wavelength at all wavelengths less than 590 mμ.

Though tritanopia is usually regarded as a rare condition, a search initiated by W. D. Wright involving the extensive publication of test colour charts resulted in a fairly large number of cases being discovered and investigated. The incidence of tritanopia has been found to be of the order of 1 in 20,000 persons. The indications are that tritanopia is an inherited condition but its incidence amongst women and men is not so widely different as in the cases of the more commonly observed types of defective vision. The consequences of the absence of xanthophyll in the retina indicated above are in general agreement with the findings of W. D. Wright. It was, however, noticed that while the power of hue discrimination virtually disappears in the blue-green wavelengths, it shows a rather surprising recovery near the far-violet end of the spectrum. This would seem to suggest that in the spectral region where the absorption of xanthophyll becomes very small, the other visual pigments ferroheme and ferriheme, play a not wholly negligible part in colour vision and colour discrimination.

THE PERCEPTION OF LIGHT AND COLOUR AND THE PHYSIOLOGY OF VISION

Part VII. General Summary

By Sir C. V. Raman

(*Memoir No. 125 of the Raman Research Institute, Bangalore-6*)

Received December 10, 1960

1. Observations of the Retina

A simple but extremely powerful technique has been devised which enables an observer without any instrumental aid to see his own retina and study its functioning in varied circumstances. The observations made by this method have enabled important conclusions to be arrived at regarding the constitution of the retina and the mechanism by which light and colour are perceived. The observer sits facing a brightly lit white screen and views it through an appropriate colour filter held in front of his eye. After a sufficient interval of time, he fixes his vision on some particular point on the screen and then removes the filter. An enormously magnified picture of the retina then appears on the screen, the nature of which depends very much on the particular colour filter used. The explanation of the phenomenon is that the rays of the spectrum which in the first instance are absorbed by the filter, suddenly impinge on the retina when the filter is removed, and excite localised sensations over its different areas. These sensations project themselves on the observing screen as an enlarged image of the retina.

By correlating the absorption spectra of the filters used with the pictures of the retina perceived by the observer, it has been ascertained that the retina contains three visual pigments whose absorption spectra lie in different regions of the spectrum: Pigment A has an absorption lying in the spectral range 4000 to 5000 Å. Pigment B exerts an extremely powerful absorption in the wavelength range between 5000 and 6000 Å, while pigment C exerts a moderately powerful absorption in the region from 6000 to 7000 Å, but its absorption also extends into and partly overlaps the region covered by pigment B.

2. The Role of the Visual Pigments

The nature and properties of light when correlated with the observed facts concerning the perception of light and colour enable us to determine

the role played by the visual pigments in the retina. Each different monochromatic light in the spectrum represents radiation characterised by a different energy-quantum; the magnitude of the quantum increases progressively from the red to the violet end of the spectrum. It is a fact also that the colours observed in the spectrum change progressively and continuously from one end of it to the other. Some 250 different colours can be perceived over the whole range. A change of only one half of one per cent. in the magnitude of the light-quantum incident on and absorbed by the retinal pigments is usually sufficient to make an observable change of perceived colour, while in some parts of the spectrum a much smaller difference is thus detectable.

Monochromatic radiation is the fundamental entity with which the physicist is concerned in optics and spectroscopy. It follows from what has been stated that the fundamental visual sensations are also those excited by monochromatic radiation. The observed precision of colour perception would be inexplicable except on the hypothesis that the function of the visual pigments in the retina is to receive, absorb and then to pass on the absorbed energy-quanta to the central parts of the visual mechanism without any addition or subtraction, themselves returning to their original energy-states.

3. Identification of the Visual Pigments

The identification of the visual pigments presents no particular difficulties. In day-light vision, there is a highly pronounced maximum of luminosity in the spectrum at about 5600 Å, on either side of which the brightness falls off rapidly. It follows that pigment B of which the absorption lies in the green plays the major role in human vision, a role somewhat analogous to that which the absorption in the red by chlorophyll plays in the photosynthesis by green leaves. We can, therefore, unhesitatingly identify our pigment B with ferroheme which exhibits a powerful absorption of light in the green sector located at the same position as the maximum of visual luminosity in the spectrum.

Oxidation-reduction mechanisms play a fundamental role in the chemistry of the living structures of aerobic organisms. The recognition of ferroheme as the principal visual pigment thus automatically involves the identification of pigment C which appears in the retina in close association with pigment B as ferriheme. It is known that the absorption of light by ferriheme is weaker than that of ferroheme, but extends much further towards longer wavelengths and is indeed sensible up to the extreme red and of the spectrum. These are the properties needed for the visual pigment which functions in that region.

The pigment A which absorbs light between 4000 and 5000 Å and is the mediator of vision in the blue and violet sectors of the spectrum can be

none other than the carotenoid pigment xanthophyll which gives the characteristic golden yellow colour to the yoke of the common hen's egg. Xanthophyll finds its way into the human body through the consumption of food products containing it, and its presence in the retina is therefore not a matter for surprise. Indeed, it is the same yellow pigment which led to the anatomical name of *macula lutea* being given to the physiologically most important area in the retina.

4. COLOUR AND LUMINOSITY IN THE SPECTRUM

The known features of the absorption spectra of the visual pigments enable a satisfactory explanation to be given of the distinctive features noticeable in the different sectors of the spectrum. The very steep rise in the absorption by xanthophyll which appears in the wavelength range around 4900 Å is responsible for the rapid change in the colour of the spectrum from green to blue and the very low value of the limen of wavelength alteration needed for observable change of colour appearing in that region. The similar but less striking fall of the limen in the region around 4400 Å where the colour of the spectrum changes from blue to violet is likewise attributable to the rapid fall of the absorption by xanthophyll with diminishing wavelength appearing in that region.

The overlap in the absorption spectra of ferroheme and ferriheme between 625 mμ and 566 mμ gives rise to the appearance of yellow and orange in the spectrum as an interpolation between the green and red sectors, the yellow where the absorptions of the two pigments are of comparable strength, and the orange where the absorption by ferriheme is stronger than by ferroheme. The steep fall in the absorption by ferroheme in the same region is responsible for the limen of wavelength change needed for an observable difference of colour reaching very low values in the region around 590 mμ.

The progressive fall in the luminosity of the spectrum and the increasing limen of wavelength change for a perceptible colour difference manifested near the extreme violet and red ends of the spectrum appear as consequences respectively of the diminishing absorption and the diminishing slope of the absorption curves of xanthophyll and of ferriheme in those two regions.

Some remarkable effects are observed when a colour filter which transmits only the blue-violet part of the spectrum is held in front of the eye and a polaroid is placed and alternately taken out and put in before the filter. These effects cease to be observable when the illumination of the field under observation is diminished so as to fall below the photopic level. They afford

a conclusive demonstration that xanthophyll is the visual pigment which enables us to perceive the blue and violet colours of the spectrum.

5. Non-Homogeneous Light

The visual sensations produced by heterogeneous light result from the synthesis by the centre of the sensations excited by the monochromatic radiations of which it is composed. The results of the synthesis may be either a chromatic or an achromatic sensation. Chromatic sensations arise when only two of the three visual pigments function, as for example when the radiations from the extreme red and violet ends of the spectrum are superposed, giving rise to the purples, or when the superposed radiations lie in the spectral range where xanthophyll has no absorption, *viz.*, in the red and green sectors; the resultant sensation is then a pure spectral colour.

The achromatic sensation arises when all the three visual pigments function in appropriate strengths. This enables a satisfactory explanation to be given of the fact that in a whole series of cases, the superposition of only two monochromatic radiations of appropriately chosen wavelengths results in the complete abolition of colour. In general, the sensations excited by heterogeneous light are a mixture of the chromatic and achromatic sensations which may be regarded as independent effects. The fact that all observable colours may be regarded as a superposition in appropriate proportions of white light and a pure spectral colour (including the pure purples) thus receives a satisfactory explanation.

The results obtained in experiments in which a pure spectral colour is sought to be reproduced by the superposition of other spectral colours may be interpreted in the same manner. The so-called spectral chromaticity coefficients determined in such experiments, when they have positive values, exhibit a parallelism with the absorption strength of the visual pigments which function in the respective spectral regions. *Per contra*, the appearance of negative values of the coefficients indicates that achromatic sensations are produced.

6. Defective Colour Vision

The existence of both ferroheme and ferriheme as visual pigments in the retina presupposes that there is a biochemical mechanism which determines the proportions in which they are normally present. Any deviations of the mechanism from normality would result in ferriheme being either totally absent or else being present much in excess. There would also be intermediate cases. The existence of four types of inheritable defect in colour

vision would thus be explicable in terms of the biochemical mechanism which determines the ferroheme-ferriheme ratio in the retina. These are respectively the protanopic, protanomalous, deuteranomalous and deuteranopic types of colour vision. The features of these different types of defects are readily predictable and the results thus deduced are in agreement with the observed facts. Tritanopia is likewise explicable as due to the complete absence of xanthophyll from the retina.

7. REFERECENS

The literature on colour vision and related topics is very voluminous. The under-mentioned books were found very useful by the writer. Besides containing extensive bibliographies, they gave clear accounts of the present state of knowledge in their respective fields and factual information of value:—

Bouma, P. J. .. *Physical Aspects of Colour*, Philips, Eindhoven, 1947.

Yves Le Grand .. *Light, Colour and Vision*, Chapman and Hall, London, 1957.

Goodwin, T. W. .. *The Comparative Biochemistry of the Carotenoids*, Chapman and Hall, London, 1952.

Lemberg, R. and Legge, J. W. .. *Hematin Compounds and Bile Pigments*, Interscience, New York, 1949.

The following is a selected list of papers containing factual information of importance in relation to the subject-matter of the present memoir.

Luminous Efficiency in the Spectrum

Walters and Wright .. *Proc. Roy. Soc.*, **B**, 1943, **131**, 340.

Hue Discrimination in the Spectrum.

Tyndall, E. P. T. .. *J. Opt. Soc. Amer.*, 1933, **23**, 15.

Wright and Pitt .. *Proc. Phys. Soc.*, 1934, **46**, 459.

Haase, G. .. *Ann. Physik*, 1934, **20**, 75.

Colour Mixing Experiments.

Wright, W. D. .. *Jour. Sci. Inst.*, 1939, **16**, 10.

———— .. *Trans. Opt. Soc.*, 1929, **30**, 140.

———— .. *Ibid.*, 1930, **31**, 201.

Defective Colour Vision.

Pitt, F. H. G. .. *Proc. Roy. Soc.* **B**, 1945, **132**, 101.

McKeon and Wright .. *Proc. Phy. Soc.*, 1940, **52**, 464.

Nelson, J. H. .. *Ibid.*, 1938, **50**, 661.

Judd, D. B. .. *Jour. Opt. Soc. Amer.*, 1943, **33**, 294.

———— .. *Ibid.*, 1945, **35**, 199.

———— .. *Jour. Res. Nat. Bur. St.*, 1948, **41**, 247.

Wright, W. D. .. *Jour. Opt. Soc. Amer.*, 1952, **42**, 509.

1048-60 Printed at the Bangalore Press, Bangalore City, by T. K. Balakrishnan, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

Supplement to the

"Proceedings of the Indian Academy of Sciences"

INDEX TO VOL. LII—(A)

AUTHORS' INDEX

Ambiye, Kumari, S. Y., Bhagwat, S. D., Joshi, M. C., Sharma, R. P. and Subbarao, B. N. — Decay scheme of EU^{156}, 157.

Bagavant, G. .. *See* Banerjee and Bagavant.

Banerjee, D. K. and Bagavant, G. — Isomerisations of substituted β-keto-esters, II, 165.

Bhagavantam, S. and Chelam, E. V. — Elastic behaviour of matter under very high pressures. Uniform compression, 1.

Bhagwat, S. D. .. *See* Ambiye and others.

Chaturvedi, R. K. .. *See* Ramaiah and Chaturvedi.

Chelam, E. V. .. *See* Bhagavantam and Chelam.

Iwao, S. .. *See* Sudarshan and Iwao.

Jayaraman, A. .. The luminescence spectra of diamond, 189.

Joshi, M. C. .. *See* Ambiye and others.

Kane, R. P. .. A critical appraisal of some cosmic ray data for the I.G.Y., 69.

Kane, R. P. and Thakore, S. R. — Latitude dependence of the daily variation of cosmic ray intensity, 122.

Krishnamurti, D. .. The Raman spectra of Polycrystalline solids, 20.

Lalitha Ramaswamy .. *See* Puranik and Lalitha Ramaswamy.

Mahadevan, C. .. *See* Rao and Mahadevan.

Mathai, I. M. .. The influence of substituents on the additive reactivity of ethylene derivatives, VI, 182.

Moosath, S. S. .. *See* Sosamma and Moosath.

Narasimhan, R. .. On the system and engineering design of the general purpose electronic digital computer at T.I.F.R., 47.

Prakash, Nirmala and Ram Behari	Deviations from parallelism and equidistance in Finsler space, 209.
Puranik, P. G. and Lalitha Ramaswamy	Potential constants and calculated thermodynamic properties for diiodoethylene, 135.
Radha, T. K. ..	*See* Ramakrishnan and others.
Ramachandran, G. N. ..	A problem in probability related to the passage of light through a cloud of particles, 87.
..	Analysis of the X-ray diffraction pattern of helical structures, 240.
Ramaiah, N. A. and Chaturvedi, R. K.	Conductometric determination of a few physico-chemical constants of murexide, 197.
Ramakrishnan, Alladi, Radha, T. K. and Thunga, R.	On the decomposition of the Feynman propagator, 228.
Raman, C. V. ..	The perception of light and colour and the physiology of vision, I, II, III, IV, V, VI, VII, 255, 267, 281, 292, 305, 314, 324.
Ram Behari ..	*See* Prakash and Ram Behari.
Rangaswami, S. and Rao, E. Venkata	Chemical components of *Pulmieria alba* Linn., 173.
Rao, B. D. Nageswara and Venkateswarlu, Putcha	The nuclear magnetic resonance spectra of four spin systems, I, 109.
Rao, B. N. Subba ..	Levels in CO^{59}, 130.
Rao, E. Venkata ..	*See* Rangaswami and Rao.
Rao, K. Kameswara and Mahadevan, C.	Studies on lead-zinc mineralization in Karempudi area, Guntur District, Andhra Pradesh, 143.
Sharma, R. P. ..	*See* Ambiye and others.
Sosamma Chandy and Moosath, S. S.	Studies on metallic halide dioxanates, III, 58.
Subbarao, B. N. ..	*See* Ambiye and others.
Sudarshan, E. C. G. and Iwao, S.	A model for hyperfragments, 27.
Swaminathan, S. ..	*See* Viswanathan and Swaminathan.
Thakore, S. R. ..	*See* Kane and Thakore.
Thunga, R. ..	*See* Ramakrishnan and others.
Thyagarajan, G. ..	*See* Venkateswarlu and Thyagarajan.

Venkataraman, S. .. Studies on collagen, VI, 80.

Venkateswarlu, K. and Thyagarajan, G. Potential constants of ethylene oxide, ethylene oxide-d_4 and ethylene sulphide, 101.

Venkateswarlu, Putcha .. *See* Rao and Venkateswarlu.

Viswanathan, K. and Swaminathan, S. *dl*-Marindinin (dihydrokawain) and some related 6-aryl-5, 6-dihydro-4-methoxy-2-pyrones, 63.

Viswanathan, K. S. .. The Dirac equation for many-electron systems, 35.

TITLE INDEX

CO^{59}, levels in (Rao), 130.

Collagen, studies, VI (Venkataraman), 80.

Cosmic ray data for the I.G.Y., some, a critical appraisal of (Kane), 69.

Cosmic ray intensity, latitude dependence of the daily variation of (Kane and Thakore), 122.

Elastic behaviour of matter under very high pressures. Uniform compression (Bhagavantam and Chelam), 1.

Electronic digital computer at T.I.F.R., the general purpose, on the system and engineering design of (Narasimhan), 47.

Ethylene derivatives, additive reactivity of, the influence of substituents on, VI (Mathai), 182.

Ethylene oxide, ethylene oxide-d_4 and ethylene sulphide, potential constants of (Venkateswarlu and Thyagarajan), 101.

EU^{156}, decay scheme of (Ambiye and others), 157.

Feynman propagator, on the decomposition of (Ramakrishnan and others), 228.

Finsler space, deviations from parallelism and equidistance in (Prakash and Ram Behari), 209.

Hyperfragments, a model for (Sudarshan and Iwao), 27.

Isomerisations of substituted β-keto-esters, II (Banerjee and Bagavant), 165.

Diiodoethylene, potential constants and calculated thermodynamic properties for (Puranik and Lalitha Ramaswamy), 135.

Dirac equation for many-electron systems (Viswanathan), 35.

Lead-zinc mineralization in Karempudi area, Guntur District, Andhra Pradesh, studies, 143.

Light, passage of, through a cloud of particles, a problem in probability related to the (Ramachandran), 87.

Luminescence spectra of diamond (Jayaraman), 189.

dl-Marindinin (dihydrokawain) and some related 6-aryl-5, 6-dihydro-4-methoxy-2-pyrones (Viswanathan and Swaminathan), 63.

Metallic halide dioxanates, studies, III (Sosamma Chandy and Moosath), 58.

Murexide, conductometric determination of a few physico-chemical constants of (Ramaiah and Chaturvedi), 197.

Nuclear magnetic resonance spectra of four spin systems, I (Rao and Venkateswarlu), 109.

Physiology of vision, the perception of light and colour and the, I, II, III, IV, V, VI, VII (Raman), 255, 267, 281, 292, 305, 314, 324.

Pulmieria alba Linn., chemical components of (Rangaswami and Rao), 173.

Raman spectra of polycrystalline solids (Krishnamurti), 20.

X-ray diffraction pattern of helical structures, analysis of (Ramachandran), 240.

NOTICE TO AUTHORS

Scientific papers intended for publication in the *Proceedings of the Indian Academy of Sciences* can be accepted only when they are communicated by a Fellow of the Academy whose duty shall be to satisfy himself that such communications are fit to be read at the Meeting of the Academy and published in its *Proceedings*.

Papers should not ordinarily exceed fifty pages of foolscap. MSS. should be either typewritten or written in legible hand on one side of the paper. All papers should be carefully revised by the authors and should be absolutely in final form for printing. Position for text-figures should be indicated. Each paper shall conclude with a critical summary not exceeding 350 words.

Drawings, diagrams or other illustrations should be made on larger scale (preferably) twice the size than the ones in which they are intended to appear. They should be done in Indian ink on bristol board with lettering in pencil. Scale of magnification of camera lucida tracings should be indicated by the side of drawings. In certain special cases arrangements will also be made for monochrome lithographic and other colour plates. Reduction of illustrations desired should be indicated in pencil. Appropriate legends should accompany all drawings. Names of authors are to be marked in pencil on the left-hand corner of drawing sheets. Photomicrographs should be securely mounted with colourless paste.

All tables, quotations and footnotes which will be set hereafter (beginning from Vol. I, No. 2) in types smaller than the text, should be typewritten on separate sheets and placed with the text in proper sequence. Footnotes should be numbered in Arabic numerals.

References to literature in the text should be given, whenever possible, in chronological order, only the names of authors and years of publication, in brackets, being given. They should be cited in full after the summary, the authors' names following in alphabetical order. Thus,

Name or Names of author; Name of Journal (abbreviation) with a single underline; Year of publication; Number of Volume with a double underline, and lastly page. The following would be a useful illustration:—

Bergmann and Stather . . . . . Z. Physiol. Chem., 1926, 152, 189.

Two copies of slip-proof and wherever possible, a page proof for final revision will be sent to authors. All corrections are best made on the slip-proof which should be transmitted to the Office of the Academy. All proof corrections involve heavy expenses which would be negligible if the papers are carefully revised by the authors before submission.

Fifty free reprints including plates and with cover will be supplied for each paper. Additional copies can be supplied at cost on previous intimation.

Blocks appearing in the *Proceedings* will be available for purchase by their respective authors. Orders for the same should be sent along with the corrected proofs and in any case not later than one month after the date of publication of the paper. The price charged would be 25% of the actual cost of the blocks *plus* freight and despatching charges. If the blocks are reproduced in other journals or publications, due acknowledgment should be made in them to the *Proceedings*.

The original drawings and plates of blocks appearing in the *Proceedings* will be returned to such of the authors as may require them provided the cost of despatching such originals is borne by them.

CONTENTS

		Page
The Perception of Light and Colour and the Physiology of Vision. Part I. The Mechanism of Perception	Sir C. V. Raman	255
The Perception of Light and Colour and the Physiology of Vision. Part II. The Visual Pigments	Sir C. V. Raman	267
The Perception of Light and Colour and the Physiology of Vision. Part III. The Carotenoid Pigment	Sir C. V. Raman	281
The Perception of Light and Colour and the Physiology of Vision. Part IV. Ferroheme and Ferriheme	Sir C. V. Raman	292
The Perception of Light and Colour and the Physiology of Vision. Part V. The Colour Triangle	Sir C. V. Raman	305
The Perception of Light and Colour and the Physiology of Vision. Part VI. Defective Colour Vision	Sir C. V. Raman	314
The Perception of Light and Colour and the Physiology of Vision. Part VII. General Summary	Sir C. V. Raman	324

#480